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PATENT- OG VAREMÆRKESTYRELSEN

Title: MICRODEVICE AND METHOD FOR SAMPLING AND CONCENTRATION OF PARTICLES FROM BIOAEROSOLS

Prior art:

Mainelis, A. et al. Collection of airborne microorganisms by a new electrostatic precipitator. *Journal of Aerosol Science* 33 (10):1417-1432, 2002.

Mainelis, A. et al. Design and collection efficiency of a new electrostatic precipitator for bioaerosol collection. *Aerosol Science & Technology* 36 (11):1073-1085, 2002.

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US20020017195 2002: Tolvanen, J. K., Electric filter

US6623544 2003: Kaura; Kamaljit S Air purification system and method of operation

FIELD OF THE INVENTION

The invention relates to a device for collecting biological agents from air.

BACKGROUND OF THE INVENTION

In order to facilitate rapid detection of airborne pathogens capable of causing either natural or deliberate epidemics, it is of utmost importance to collect particles containing biological material directly from air in a form suitable for further analysis. Typically, particles containing or consisting of one or more biological organisms (bioparticles), are captured by passing (samples of) air through porous filters. To size fractionate for selected ranges of particles, a succession of filters have been used to select for the right size particles which are subsequently collected at - and can be cultured on growth media plates.within the device (The Andersen sampler). When bioparticles contain live organism, they can give rise to growth (colonies) on the media plate, which can be collected and analyzed easily. However, the researcher will have to wait for the colonies (colony forming units or CFU) to become visible, which, depending on the cultured organism, can take from days to weeks. Additionally, the possibility exists that a biological organism of interest might not thrive on the growth media. It is also possible that particles of interest are captured within or adhere to the filtering system and therefore go un-detected. To compensate for sample loss a large volume of air need to be processed through the system. A complimentary method, which can be used in the combination with the filter sampling, is to maximize the number of particles in a given volume of air, by using e.g. cyclones or other vortex type air samplers that facilitate an initial concentration of particles. The later technique has also been used to concentrate particles into a volume of liquid, giving immediate access to captured bioparticles for a variety of different microbiological, biochemical and molecular analyses. Funneling particles into a liquid is associated with heating and evaporation of the liquid and often volumes of liquid (over 1 ml) are required to prevent the system from drying out during the capture process. Subsequently, the liquid can be processed by centrifugation to perform a final concentrate of the sample.

The present invention describes the development of a device and a method that allows for large volumes of air to be processed, without submitting the collected sample to either a media or a liquid, in order to maximize the yield and minimize the number of concomitant steps to perform the optimization of the sample collection into a concentrate. The invention describes a sample collection system based on electrostatic precipitation performed in a microstructure that allows for flows of up to several hundred milliliter air per minute. The device is capable of capturing bioparticles with

very high efficiency and at the same time it performs the capture on a dry surface in a form suitable for various subsequent analyses.

INTRODUCTION

Sampling of bioparticles (particles containing or comprising living microorganisms) can be done by air to air, air to surface or air to liquid methods. Liquid methods can be cumbersome because frost effects at temperatures below 0°C and compressing air is a problem for further analysis. Key aspects of the sampling are sampling volume, capture efficiency and concentrating efficiency of the sampling technology. A standard cyclone is capable of taking in one m^3 of air per second and concentrating captured particles in 1-2 ml of fluid, which is a large volume in biochemical analyses. A volume in this range would fill a 96 well plate and consume reagents equivalent to approximately € 14 per plate per second. It is obvious that the rapid air sampling by the cyclone quickly is compromised by the subsequent sample preparation. Thus, the optimal system would require a direct sampling and concentration step in the same process. There is a trend toward new sampling technologies that allow for sampling of airborne bioparticles at much lower flow rates than what has been previously available (Vincent et al 1999). This invention describes the novel capability of combining the sampling and concentration of particles in a one-step process within a micro-structured device. The identified mechanism is based on electrostatic precipitation of charged bioparticles, which has recently been described as a excellent technology for sampling bacterial spores from air (Mainelis et al 2002b). In the invention, the principles of electrostatic precipitation are embodied it into a microstructure, which shows an excellent capture efficiency of up to 80% as compared to the generally achieved 50% (e.g. the BioGuardian Air Sampler supplied by Tekkie, Washington, USA see http://www.tekkie.com/docs/BioGuardian.pdf). The invention successfully combines the difficult task of capturing and concentrating air samples in one experimental procedure. Micro-scale electrostatic precipitation in a small biochip is a major advantage compared to existing sample capture technology. The combined compact design of the invention is therefore the most efficient form of methodology for performing subsequent analysis on biological warfare agents (e.g. DNA analysis). Further details are described in the section containing the theoretical basis and modeling of our sampling technology.

THEORY

Electrostatic precipitation of airborne biological particles has been utilized for sampling of bacteria onto agar plates in conventional devices (here meaning devices not based on microfabrication) (Mainelis et al 2002a;Mainelis, Willeke, Adhikari, Reponen, & Grinshpun 2002b;Mainelis et al 2004) and for decontamination of a dental practice (Iversen & Tolo 1975). When released as bioaerosols, bacterial spores from *Bacillus subtilis* var. *niger* (utilized as model organism for *Bacillus anthracis*) have been shown to carry a substantial electrostatic charge that decreases over time rendering a population with a substantial spread of carried electrostatic charge (Mainelis, Adhikari, Willeke, Lee, Reponen, & Grinshpun 2002a). It has also been demonstrated that recharging can occur by passing the bioaerosol over an ionizer (Mainelis, Willeke, Adhikari, Reponen, & Grinshpun 2002b). Thus, the sampling of bioaerosols using electrostatic fields is a well known and proven technology. However, the present invention discloses the utilization of the electrostatic sampling technology in a microfabricated device in such a way that biological particles can be concentrated from (an aliquot of) air. This is an important finding since the currently used liquid-based microfabricated devices by nature are only capable of handling liquid sample sizes in the microliter range. Therefore, microfabricated devices are not suitable for handling liquid samples harvested from e.g. a cyclone producing 1-2 ml/s. However, microfabricated devices are useful as bioaerosol sampling technology, if the sampling of bioparticles is done directly in the microsystem. This can be achieved by utilizing electrostatic techniques to concentrate biological particles from an air stream passing a microfabricated channel. At any given pressure air can be passed much more easily through microfabricated devices than a liquid. This is due to the resistance of flow is being defined as the viscosity of the medium (1.0×10^{-3} N·s/m² for water at 20 °C and 1.8×10^{-5} N·s/m² for air at 20 °C), which therefore will allow a much larger flow of air than fluid through a capillary.

Design considerations of microfabricated device

In order to find the most effective design for capturing bioparticles by means of an electrostatic field, we have compared the biophysical modeling of three different types of design shown below.

Model 1 (See Figure 1) was based on four pointed electrodes placed in the top of the channel and an electrically grounded capture area (common for all models). The capture area could represent a localized area on-chip on which the concentration of

bioparticles would occur. This model is referred in the following text and figures as "Point Electrode"

Model 2 (See Figure 2) was based on a wire transecting the entire channel and thus providing a more uniform potential along the length of the channel. The electrically grounded capture area was similar as model 1 and model 3. This model is referred in the following text and figures as "Wire Electrode"

Model 3 (See Figure 3) was an extension of Model 2 in which a uniform potential was achieved in both length and width of the channel by using the entire top area of the channel as electrical conductor. The electrically grounded capture area was similar to model 1 and 2. This model is referred in the following text and figures as "Plate Electrode"

Simplified model

The optimization modeling problem is truly multi-dimensional since there had to be a non-linear interaction of several independent and dependent parameters, as given by

a) channel, electrode and capture area geometry
b) spore properties as size, density, initial charge
c) air flow rate
d) applied potential and direction of field lines.

In order to obtain an estimate for the idealized capture efficiency, firstly a simplified theoretical analysis was performed assuming uniform velocity of all particles, in viscid fluid, uniform field and field charging of spores (illustrated on Figure 4). In this way an expression is obtained for the idealized capture length given by:

$$L_c = \sqrt{\frac{\rho_p d_p H \xi}{18 \varepsilon W^2 V^2}}$$

Equation 1

Where:

W	=	channel width
ε	=	electrical permittivity of air
ρ_p	=	spore material density
d_p	=	spore size
ξ	=	air flow rate

The CFD +ACE simulation tool was utilized to setup the simplified model to find an estimate for the minimum applied potential for which all spores would be captured.

The following are constants and set as fixed parameters:

Channel length	10 mm
Channel height	3 mm
Channel width	3 mm
Capture area	Entire bottom surface of channel
Spore size	Uniform distributions of 1, 2, 3, 4, 5, 6 mm
Spore density	1050 kg/m^3 (Nominal for biological particles)
Electrical permittivity	$8.85x10^{-12}$ F/m
Gravitational acceleration	9.81 m/s^2

And the following two parameters were set as variables:

Air flow rate	0.1, 1, 10 l/min
Applied potential	50, 100, 200, 500, 1000 V

The modeling resulted in:

At 0.1 l/min all spores will be captured at an applied potential of 50 V

	Applied potential (V)				
Spore size (μm)	50	100	200	500	1000
1	capture	capture	capture	capture	capture
2	capture	capture	capture	capture	capture
3	capture	capture	capture	capture	capture
4	capture	capture	capture	capture	capture
5	capture	capture	capture	capture	capture
6	capture	capture	capture	capture	capture
Ideal capture efficiency	100 %	100 %	100 %	100 %	100 %

At 1.0 l/min all spores will be captured at an applied potential of 200 V

	Applied potential (V)				
Spore size (μm)	50	100	200	500	1000
1	no capture	capture	capture	capture	capture
2	no capture	no capture	capture	capture	capture
3	no capture	no capture	capture	capture	capture
4	no capture	no capture	capture	capture	capture
5	no capture	no capture	capture	capture	capture
6	no capture	no capture	capture	capture	capture
Ideal capture efficiency	0 %	17 %	100 %	100 %	100 %

5 At 10.0 l/min all spores will be captured at a an applied potential above 1000 V

	Applied potential (V)				
Spore size (μm)	50	100	200	500	1000
1	no capture	no capture	no capture	no capture	capture
2	no capture	no capture	no capture	no capture	no capture
3	no capture	no capture	no capture	no capture	no capture
4	no capture	no capture	no capture	no capture	no capture
5	no capture	no capture	no capture	no capture	no capture
6	no capture	no capture	no capture	no capture	no capture
Ideal capture efficiency	0 %	0 %	0 %	0 %	17 %

This analysis provided a series of key results, being a set of threshold potentials for which 100% capture would be possible at different flow rates. It was also found that the electrostatic acceleration was inversely proportional to spore size. Furthermore, 10 the capture length for small size spores is shorter than for large spores. Finally, the effect of gravity was negligible for the range of parameters used. The model did however incorporate a set of limitations such as a probable disability to estimate the exact value of the idealized capture efficiency since viscous effects had been excluded from the model. A uniform slow assumption was implied and excluded contributions 15 from spatial variations in the flow field within the channel. Additionally, a uniform

electrical field was assumed for to the "Plate Model", but not to the "Wire" or "Point Electrode" models. In order to compare the capture efficiency of the different models a multi-dimensional model based on the results from the simplified model was set up.

5 Multi-dimensional model

The results from the simplified model in specifying a range of voltages and flow rates were used for which considerable capture efficiency would be expected. These settings were applied to all three design models. The fixed parameters were as follows:

Channel length	10 mm
Channel height	3 mm
Channel width	3 mm
Capture area length	5 mm
Capture area width	2 mm
Spore size	Uniform distribution of 1, 2, 3, 4, 5, 6 mm
Spore density	1050 kg/m^3
Air flow rate	1 l/min

10 The following parameters are variables:

Electrode configuration	Plate, 1 Wire, 10 Point, 2 Wire, 2 Point
Applied potential	100 – 1000 V

Air was injected with a parabolic inlet velocity profile and 1000 spores were distributed randomly over the opening. The velocity profile at the opening of the channel is shown in Figure 5 with concomitant contour lines for the corresponding voltage profile of the
15 given design model. The simulation gave following results shown in Figure 6. The left side shows the voltage profile for the given design model and the right side shows the individual spore trajectories for each of the 1000 spores entering the opening. It is apparent that the "Plate Electrode" design deflects the spore trajectories the most.

20 The deflection of the spores is better illustrated with a depletion plot (See Figure 7a) where representative slides are cut out as plate sections along the length of the channel. The color intensity represents the spore concentration.

Plate Electrode design. The spore concentration is shown as a normalized color map (0-1). The leftmost slide at the back of the plot illustrates the entrance of the spores,
25 whereas the rightmost slide at the front depicts the exit point of the channel. The plot

shows the concentration profile of the spores at each slide location. A clear depletion (zero concentration) is shown at the top of the channel.

Wire Electrode design. The wire design is illustrated in Figure 7b where a clear depletion (zero concentration) is shown at the top of the channel along the length of the wire. The effect is not as prominent as observed with the Plate Electrode design (see Figure 7a).

Point Electrode design. The Point Electrode design, shown in figure 7c, resulted in depletion (zero concentration) localized around the point electrodes. The effect is small compared to both the Wire Electrode and Plate Electrode designs.

In order to understand the results from the depletion plots it is useful to study the spore trajectory along the electrostatic field in the dimensions height and length. The plots in Figures 8a-8c illustrate the path the spores follow from top to bottom of the channel.

Figure 8a shows the Plate Electrode Design. The plot shows the height-length trajectory of the spores in the channel. According to the model, the spores will follow the electrostatic field in the orthogonal direction. This design gives a very effective movement of spores from top to bottom, which makes the spores travel the shortest distance from their location in space to the capture surface.

Figure 8b shows the Wire Electrode Design. The plot shows the height-length trajectory as explained above. Spores move orthogonally in the electrostatic field. According to travel distance this design seems less effective than the plate design. If the wire was the site of capture rather than the bottom plate (this depend on initial charge on the spore) a large concentration of spores could be achieved at the wire.

Figure 8c shows the Point Electrode Design. The plot shows the height-length trajectory as explained above. Spores move orthogonally in the electrostatic field. The point electrode makes the effect high in the close vicinity to the point and small at distance. The point electrode design should therefore be excellent in concentrating spores at a certain point and could be used in combination with other types of electrostatic precipitation designs.

Following the evaluation of the different types of designs and their effectiveness in capturing spores, the effects at different voltages was explored and the results from the different designs compared. The modeling results depicted in the previous figures illustrate that a point electrode design is the most inadequate, a wire electrode design

the second best and a plate electrode the superior design in terms of capture efficiency for a microfabricated channel. Figure 9 illustrates the theoretical capture efficiency calculated from the ratio of numbers of spores impacting the capture area to total number of spores released into the channel of the three different designs as a function of voltage. It can be seen that there is an improvement in capture efficiency from a point electrode (10 points) to a wire design and again to a plate design. Figure 10 illustrates the improved capturing effects of introducing a two wire design compared to a one wire design. The effect approaches the effect of the plate design.

It is apparent that increasing the number of electrode points increases the capture efficiency, as the point electrode design approaches the wire design (see Figures 11 and Figure 12). A two-point electrode design was utilized to generate data for a comparison with the Point electrode design (10 points) used in the previous analysis. A two point electrode design was found to be less effective than a 10 point design in capturing spores. The results obtained from; firstly the simplified model and secondly from the multidimensional model, strongly suggest that a plate design is the best for capturing spores in a microfabricated channel. Although the modeling is done with a set of assumptions representing unknown parameters, these parameters are not going to reflect the relative relationship between the capture efficiencies but only the absolute numbers. It is therefore evident that the plate design will achieve the maximal capture efficiency of spores. Other designs might be better for concentrating particles at a given location as shown for the wire and point designs.

Methods and materials for experimentation

The CFD +ACE software used to model the behavior of spore particles in applied electrostatic fields using different electrode designs was obtained from CFD Research Cooperation, Huntsville, AL, USA. Statistical Data analysis was performed using GraphPad Prism version 3.03 for Windows XP (GraphPad Software Inc. USA). Various data manipulations were done using Microsoft Excel 2002 (Service pack 2).

The GPIB software for controlling the voltage generator (see below) used for capturing spores on-chip in the laboratory setup was specifically developed by RTX Telecom A/S, Nørresundby, Denmark. The particle size analyzer model 3321 from TSI (TSI Inc., Shoreview, MN, USA) was controlled by the TSI software Aerosol Instrument Manager. The Fenix Laser was controlled using Winmark Pro version 4.0.0 build 3773 (Synrad software, Mukilteo, WA USA). The lasing processes were run at 1200 dpi with a speed of 400 pulses/sec and power intensities in the range of 5-10%. Dependent on the required channel depth, the number of overlapping markings was set to a number in between 1 to 10 (10 gave deep channels, whereas 1 gave a very superficial ablation

of 20-30 µm deep). Very deep channels (>800 µm) tend to become concave in shape due to the loss of focus of the laser as the ablation digs into the material. The GPIB controlled high voltage power supply model PS310/1250V-25W was purchased from Stanford Research Systems (Stanford Research Systems, Inc. Sunnyvale, CA, USA). The power supply was utilized for generating voltages in the range of 100-1200 V. The voltage and pump control of the EP-chips used in the experiments performed in the 63 m^3 stainless steel encapsulated aerosol room were developed by RTX Telecom A/S, Nørresundby, Denmark. The step-up converter delivered 250 V from four 1.5 V standard AA batteries and supported the air pump with power. A vacuum pump model DC06/03f from Fürgut (Erich Fürgut, Tannheim, Germany) was utilized to mediate air suction in the EP-chips. The DC06/03f device operated at a voltage of 6 V utilizing 220-330 mA, delivering 1.0 l/min of air against a maximal pressure difference of 180 mbar, and had a total weight of 30 g. The advantages of the pump were following: small size, lightweight, pulsation-free flow, reversible flow of the medium by changing motor polarity, flow volume adjustable by controlling voltage. The Model 3321 Aerosol Particle Sizer (3321 APS) (TSI Inc., Shoreview, MN USA) provides two measurements: aerodynamic size and relative light scattering intensity. It detects particles in the 0.37 to 20 µm range with high resolution sizing from 0.5 to 20 µm in aerodynamic diameter. The instrument measures the aerodynamic size in real time. The instrument was set to an air flow of 5 l/min.

The aerosol chamber utilized for the lab experiments was kindly provided by The Institute of Occupational Health, Copenhagen, Denmark and manufactured by Mikrolab (Mikrolab Aarhus A/S, Højbjerg, Denmark). The 50 liter chamber was made of stainless steel mounted on a rotor arrangement allowing the entire chamber to rotate at a speed of 0-60 rpm. The core chamber could be separated in tree parts (two ends and one cylinder). Both end parts were fastened securely by 5 handles. Before assembly the spore material was positioned in the cylinder. The chamber was open and a pump system could blow air with controlled temperature and humidity through the chamber from the backside of the chamber with exit at the nozzle shown on picture 13. The nozzle could be connected to a two-piece metal tubing (see picture 13) that split the air stream into two. One part going to a HEPA filtered output and the other and smaller entering the chip. PMMA chip connected to the two-piece metal tubing interfaced to the output nozzle from the aerosol chamber. The metal tube seen as turning to the upper right side of the picture entered a low-pressure tract equipped with two overlapped Whatman qualitative filter papers Grade 2V pore size 8 µm (Whatman International Ltd., Maidstone, Kent UK).

A capture channel created by microscope slides interfaced to the two-piece metal tubing and connected to the aerosol chambers exit nozzle (see figure 14). The

interfacing was done by 0.5 mm inner diameter Teflon tubing. The Teflon tubing was glued to the glass by a two component epoxy and hardened overnight. Flow, temperature and humidity control allowing a controlled amount of air at a certain temperature and humidity to enter the aerosol chamber. The air temperature was 26°C and the humidity was 50%.

The Fenix Laser model 48-2 a 25 watt RF-excited pulsed CO_2 laser (Synrad, Inc. Mukilteo, WA USA) equipped with a 80 mm lens (lens focal length) with a nominal field of 27x27 cm and a working distance of 74 ± 1 cm. The spot size was 116 µm. A CO_2 gas mixture provides an output wavelength at or near 10.6 µm. The Fenix Laser lens system uses a flat field principle giving a high quality and uniformity in the marking process. Rapid Thermo bonding (see below) of the PMMA parts were done at temperatures from 120-200 °C in a BD heating oven (Binder GmbH, Tüttlingen, Germany).

Consumables

PMMA (Poly-methyl-meth-acrylate) plates (Riacryl, RIAS A/S, Roskilde, Denmark) are transparent (>90% transmission), have an excellent UV stability, low water absorption and high abrasion resistance. PMMA has been utilized for making microstructures with laser ablation (Johnson et al 2001) and the material is chemical inert to the ablation process compared with other materials as poly(ethylene-terephthalate-glycol), poly(vinylchloride), and poly(carbonate) (Pugmire et al 2002). The material has furthermore proven suitable to build complicated integrated microfluidic systems characterized as lab-on-chip systems (Johnson, Badr, Barrett, Lai, Lu, Madou, & Bachas 2001). The material expresses little joule heating when exposed to electrical fields and can be utilized for DNA separation (Chen & Chen 2000;Sung et al 2001).

Different PMMA designs were made and successfully annealed to each other by rapid thermo bonding at 160-200°C. Alignment was achieved by a prototype alignment tool developed by Mikrolab Aarhus A/S and the alignment was secured by adhesive bonding prior to the rapid thermo bonding by positioning 0.2-0.5 µl cyanoacrylate at the corners of the PMMA plates. It was found that minor amounts of adhesive did not interfere with the thermo bonding process in the vicinity of the active micro structured parts if the adhesive bonding were kept remote to the micro structured sites. The high temperature rapid thermo bonding removed the need for thermo fusion bonding which was seen to damage both alignment and the microstructures themselves.

Electrical conductive coatings were made using Electrodag 1415M containing silver particles in 4-methyl-penthan-2-one. The conductive fluid was diluted 1:1 with acetone to reduce the thickness of the coatings.

SUMMARY OF THE INVENTION

The invention encompasses a method and a microstructure aimed at facilitating the collection and concentration of bioparticles (particles containing or comprising living or non-living microorganisms) from an air sample.

The purpose of the present invention is to perform an efficient and rapid sampling of bioparticles. This purpose is obtained by a method and a structure comprising the combined usage of an electric field induced over the air flow. Said usage consists of a structure encompassing a set of electrodes exerting an electrical field that can be varied in voltage and applied for a variable time, and a set of optimal parameter settings for the said usage

The present invention describes a method and a device that enables the application of an electrical field at an angle or perpendicular to the air-flow passing through the device and as a result of this method bioparticles are collected and concentrated within said device.

DETAILED DESCRIPTION OF THE INVENTION

This section provides a detailed description of the invention and its application in collecting and concentrating microorganisms from aerosols. More specifically in collecting and concentrating endospores of bacteria from the *Bacillus* group.

As shown in the accompanying drawings, the present invention relates to a method, device and system for sampling and concentrating microorganisms in an air sample. Said method and system is enabling the detection of said microorganism by subsequent appropriate means. The detecting step may be carried out in suitable manners, with suitable detecting means, as apparent to a person skilled in the art.

Broadly described, the sampling and concentrating methods according to the present invention is essentially concerned with the trapping or capturing by means of an electrical field applied at an angle or perpendicular to the air-flow circulated through the device.

As shown in the accompanying figures, the device comprises a chamber and two opposite positioned electrodes, preferably plate electrodes. The air sample to be examined is passed through the chamber and, as better shown in figures 15a and 15b, the chamber has an inlet for receiving the air sample into the chamber and an

outlet for releasing the air sample from the chamber, the air sample circulating through the chamber from the inlet to the outlet thereof. As also shown, the electrodes are positioned within the chamber between the inlet and the outlet, said electrodes having an electrical potential thus allowing the air sample to pass through while capturing microorganisms from it.

According the first preferred embodiment of the invention, as better shown in figs. 3, 5, 6, and 7a the electrodes consist of an assembly having two plate electrodes positioned perpendicular to the air-flow circulated through the device.

According the second preferred embodiment of the invention, as better shown in figs. 2, 5, 6, and 7b the electrodes consist of an assembly having a wire electrode and one plate electrode positioned perpendicular to the air-flow circulated through the device.

According the third preferred embodiment of the invention, as better shown in figs. 1, 6, 6, and 7c the electrodes consist of an assembly having one ore more point electrodes and one plate electrode positioned perpendicular to the air-flow circulated through the device.

In either case, according to the present invention, said device is used within a system for detecting the presence of microorganisms in an air sample, taken from either a gaseous environment to be examined, as apparent to a person skilled in the art.

According to the present invention, the system and the components thereof are preferably devised to sample and concentrate microorganisms such as bacteria (e.g. *Bacillus* spp., *Clostridium* spp., *Legionella* spp., *E. coli* O157:H7, *Neisseria* spp., *Mycobacterium tuberculosis*), fungi (e.g. *Aspergillus flavus, fumigatus, Niger, Histoplagma capsulatum, Coccidioides imitis*), viruses (e.g. small pox, influenza virus, rubella virus) and the like.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENT

A first embodiment of the present invention is illustrated in figures 3, 5, 6, and 7a. These figures show an example of the device acting as an air-monitoring unit. The unit shown in figure 3 and exemplified in figures 15a and 15b is particularly useful for sampling and concentrating harmful microorganisms that might be present in the air environment of facilities such as hospitals, schools, industries, houses, public

buildings, farms and the like. In operation, the air-sampling device is preferably operatively connected in a continuous manner to sample the air of the corresponding facility to be inspected.

The air-sampling device preferably includes an air inlet, an inlet valve, housing, a power supply, an outlet valve, an air pump, an air outlet, a control circuit board (not shown), relay wires from the different components of the system to the control board, and supports for the housing and other components of the system.

The air-sampling device typically functions as follows: the pump provides a particular aspiration flow rate ranging preferably from about 5-1000 milliliters per minute (ml/min) into the air inlet and then, into the sampling chamber. It is worth mentioning that other suitable flow rates may be used with the present invention, as apparent to a person skilled in the art. Also, the air sample to be examined may originate from a person exhaling a breath sample containing or susceptible to contain microorganisms.

In either case, whether the air sample to be examined comes from a gaseous environment or person exhaling a breath sample containing or susceptible to contain microorganisms, it is directed to the sampling chamber of the device by means of the air inlet. Once microorganisms are sampled and concentrated, a skilled person familiar with diagnostic systems will easily find various arrangements to detect the sampled microorganisms leading to identification of said microorganisms.

Preferably, the device is fabricated from the group of materials consisting of polymers, silica, glass, metals, and ceramics.

EXAMPLES

The following examples are illustrative of the wide range of applicability of the present invention and are not intended to limit its scope. Modifications and variations can be made therein without departing from the spirit and scope of the invention. Although any method and material similar or equivalent to those described herein can be used in the practice for testing of the present invention, the preferred methods and materials are described.

Example 1

Sampling of *Bacillus* spores in the air

The *Bacillus thuringiensis*-based powder used in the aerosol-trials was Biobit WP (wetable powder) (Valent Biosciences, IL, USA) based on the *B. thuringiensis* subsp. *kurstaki*. Based on plating assays of dilution series, the spore concentration of the powder is 3.2x109 spores/g (i.e., colony forming units, CFU) with a density of 0.86 g/cm³ (=860 kg/m³)

In a first set of spore capture experiments two prototypes for electrostatic precipitation were manufactured as shown in figure 14. The chips were mounted as shown in figure 13 and voltages were applied using a GPIB controlled high voltage power supply model PS310/1250V-25W. The flow through the chip was recorded to 2 l/min over a 10 minute sampling period and the total particle concentration was estimated to 26 mg/m³ with a geometric mean at 1.53 ± 1.96 µm representing a total of 3304290 particles. The figures 16 through 39 show data from experiments each consisting of nine sample periods with a duration of 30 seconds. The upper graph in each figure represents the first three sample periods and are pre-controls showing the stability of the control samples in which one of the prototypes under investigation (Collector A or Collector B) is at 0 Volt. The middle graph in each figure represents the next three 30 seconds samples. These are performed with the electrical field of the prototype set to a sample (capture) voltage of 100-1200 Volt. The bottom graph in each figure represents the last three 30 seconds sample periods and is post-controls where the prototype is returned to 0 Volt. It is important to notice that the particle counter is counting the particles that are passing through the prototype. Thus, a capture of particles is illustrated by the removal of particles from the population compared to the pre and post controls.

The results showed a significant decrease in concentration of spores in the air sample when 1200 Volt (E = 1200V/mm) were applied (see figures 27 and 39). These results show that the electroprecipitation principle is applicable in a microscale device. Furthermore, the data from these series of experiments can be used to calculate the effect on the average number of particles that passes the chip (capture efficiency) as a function of voltage. When capture efficiency is plotted versus voltage, the data can be fitted to an exponential association function with a high degree of Goodness of fit (0.983) and a symmetrical distribution of residuals around the curve (see figure 40). In essence, these data show that a capture efficiency of approximately 80% is obtained when using said experimental setup.

Example 2

Sampling of *Bacillus* spores in the air

In another set of experiments, identical *Bacillus thuringiensis*-based powder as described in Example 1 was used. The microfabricated PMMA-chip used in the experiments performed in an aerosol room was designed as shown on figures 15a and 15b. The electrodes were made using electrical conductive coatings. The height (i.e., the electrode gap) was 200 µm and the length was 20 mm. A voltage of 250 V (E = 1250 V/mm) was delivered by a step-up converter utilizing power from four 1.5 V standard AA batteries. The circuit board for said step-up converter further regulated the power to the air pump (model DC06/03f from Fürgut).

The aerosol room utilized was a 63 m^3 stainless steel encapsulated room equipped with rotor aerosol dispensers and particle counters wired to externally placed computers equipped with data acquisition cards. Particle counts and size distribution of the air in the aerosol room were registered at the exact time of sampling.

The sampling devices were flushed with sterile H_2O, and dilutions plated on LB-plates (Luria Bertani substrate; 10.0 g tryptone, 5.0 g yeast extract, 10.0 g NaCl, 15.0 g agar resuspended in 1.0 liter H2O – pH = 7.0, autoclaved) incubated overnight at 30°C and CFU (colony forming units) of the right morphology was enumerated. The results are shown in the table below.

Particles collected by PMMA-chip EP8 channel 1-7:

CHANNEL	DURATION	FLOW	CFU	Bacteria	Particles 1-10 µm
	[seconds]	[ml x min^{-1}]		[per liter air]	[per liter air]
EP8-1	30	46.2	~300	13,000	318.91
EP8-2	30	45.9	209	9,100	351.53
EP8-3	30	45.3	1	44	353.37
EP8-4	30	40.3	~550	27,300	352.93
EP8-5	30	44.5	~450	20,200	369.86
EP8-6	30	45.1	3	133	371.78
EP8-7	30	44.7	3	134	360.02

The apparent discrepancies between the particle counts and the bacterial spore concentration in the aerosol are due to the bioparticle size distribution (see figures 16 – 39) – i.e., few large bioparticles may comprise hundreds of spores. However, even

though only ~25 ml of air was sampled, the microfabricated PMMA-chip is clearly able to sample bioparticles. It should be noted that three negative controls were performed (employing air suction for up to 300 seconds through a PMMA-chip with no voltage applied), with no CFU after overnight incubation.

Example 3

Specific Uses

The present invention may be used for different purposes. Specific examples include:

a) Detection or monitoring of live bacteria in the air

The invention described herein could be used for detection of living (vegetative) bacteria in the air. The device could be installed in a ventilation duct and switched on for a period of several days or weeks. The bacteria would be captured by the capture electrodes and next detected by subsequent method known to persons skilled in the art.

b) Sampling of Spores

The invention described herein could be used for the detection of spores such as *Bacillus anthracis* spores. The device could be installed in a ventilation duct and switched on for a period of several days or weeks. The bacteria would be captured by the capture electrodes and next detected by subsequent method known to persons skilled in the art.

c) Sampling of particles in the Air

The invention described herein could be used for the sampling of particles of various size using appropriate methods of counting. More particularly, it could be used for the detection of microorganisms in air ducts, ventilation systems, air purifiers, air conditioners and vacuum cleaners, the detection of microorganisms in isolation rooms, pharmaceutical and medical clean rooms, etc.

The invention described herein could also be used for the detection of living pathogens affecting patients, the device being directly connected to the mask of a ventilator for example.

As may be appreciated, the present invention is a substantial improvement over the prior art within sampling technologies in that the electrostatic precipitation can be successfully embodied onto a biochip and provides a low-cost, robust and well-controlled means for capture of bioparticles directly from air. As apparent (see figure 40), the capture efficiency within the biochip easily approaches 80% and can be pushed higher by increasing the voltage. The continued operation of an electrostatic precipitation biochip (EP-chip) provides a single capture and concentration step, which is a major improvement of state-of-art technology.

Although the present invention was primarily designed for sampling microorganisms in an air sample taken from various environments, as will be easily understood by reading the following description and as apparent to a person skilled in the art. For this reason, the expressions "air", "duct", "ventilation", "air-monitoring unit" and the like should not be taken as to limit the scope of the present invention and include all other kinds of substances with which the present invention may be used and could be useful.

In addition, although the preferred embodiment of the present invention as illustrated in the accompanying drawings comprises various components such as valves, pumps, control circuit board, etc., and although the preferred embodiments of the sampling device/system and corresponding parts of the present invention as shown consist of certain geometrical configurations as explained and illustrated herein, not all of these components and geometries are essential to the invention and thus should not be taken in their restrictive sense, i.e. should not be taken as to limit the scope of the present invention. It is to be understood, as also apparent to a person skilled in the art, that other suitable components, as well as other suitable geometrical configurations may be used for the sampling device/system according to the present invention, as will be briefly explained hereinafter, without departing from the scope of the invention.

While several embodiments of the invention have been described herein, it will be understood that the present invention is capable of further modifications, and this application is intended to cover any variations, uses, or adaptations of the invention, following in general the principles of the invention and including such departures from

the present disclosure as to come within knowledge or customary practice in the art to which the invention pertains, and as may be applied to the essential features herein before set forth and falling within the scope of the invention as defined in the appended claims.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1, Model of the capture module having four pointed electrodes placed in the top side of the channel and an electrically grounded capture area.

Figure 2, Model of the capture module based on an electrically grounded capture area and a wire transecting the entire channel and providing a more uniform electrostatic potential along the length of the channel

Figure 3, Model of the capture module based on an electrically grounded capture area. A uniform potential is achieved in both length and width of the channel by using the entire top area of the channel as electrical conductor, resulting in two plate shaped electrodes.

Figure 4, Simplified theoretical model assuming uniform velocity of all particles, in viscid fluid, a uniform electrostatic field and uniform field charging of spores. L_c is the idealized capture length given by:

$$L_c = \sqrt{\frac{\rho_p d_p H \xi}{18 \varepsilon W^2 V^2}}$$

Where:
- W = channel width
- ε = electrical permittivity of air
- ρ_p = spore material density
- d_p = spore size
- ξ = air flow rate

Figure 5, Velocity profile at the opening of the channel with concomitant contour lines for the corresponding voltage profile of the given design model.

Figure 6, Results of a simulation of different voltage profiles. The left side shows the voltage profile for the given design model, while the right side shows the individual spore trajectories for each of the 1000 spores entering the opening.

Figure 7, Deflection of bacterial spores under the influence of electrostatic fields as generated using different electrode designs. Representative profile slides are cut out as plate sections along the length of the channel. The color intensity represents the spore concentration. (a), Depletion plot of a plate electrode design. (b) Depletion plot of a wire electrode design.. (c). Depletion plot of a point electrode design.

Figure 8. Path that bacterial spores follow under the influence of different electrode designs, showing the height-length trajectory of the spores in the channel. (a) Using the Plate Electrode Design. (b). Using the Wire Electrode Design. (c). Using the Point Electrode Design.

Figure 9. Theoretical capture efficiency calculated from the ratio of numbers of spores impacting on the capture area, to total number of spores released into the channel for the three different designs as a function of voltage applied.

Figure 10. Improved capturing effects observed after using a two wired electrode design as compared to a single wire electrode design.

Figure 11. Observed increase in capture efficiency resulting from increasing numbers of electrode points.

Figure 12. Increased capture efficiency resulting from increasing numbers of electrode points.

Figure 13. Exit from the 50 l aerosol chamber. The nozzle is connected to a two-piece metal tubing splitting the air stream into two. One part going to a HEPA filtered output and the other and smaller entering the chip. The PMMA chip is connected to the two-piece metal tubing interfaced to the output nozzle from the aerosol chamber. The metal tube seen as turning to the upper right side of the picture entered a low-pressure tract equipped with filters.

Figure 14. A capture channel was created using microscope slides. The interfacing to the metal tubing was done by epoxy-gluing of a Teflon tubing (0.5 mm inner diameter).

Figure 15a. The schematic drawing of the microfabricated PMMA-chip used in the experiments performed in an aerosol room showing the dsign of the device. The electrodes were made using electrical conductive coatings. The height (i.e., the electrode gap) was 200 µm and the length was 20 mm.

Figure 15b. Picture of the PMMA-chip. The connections between the outer silver coatings and the inner electrodes were achieved through a small hole going through the PMMA and filling of said hole with conductive fluid.

Figures 16 – 39. Data from sampling experiments each consisting of nine sample periods with a duration of 30 seconds. The upper graph in each figure represents the first three sample periods and are pre-controls showing the stability of the control samples in which one of the prototypes under investigation (Collector A or Collector B) is at 0 Volt. The middle graph in each figure represents the next three 30 seconds samples. These are performed with the electrical field of the prototype set to a sample (capture) voltage of 100-1200 Volt. The bottom graph in each figure represents the last three 30 seconds sample periods and is post-controls where the prototype is returned to 0 Volt. The particle counts represents the particles that are passing through the device. Thus, capture of particles is illustrated by the removal of particles from the population compared to the pre and post controls.

Figure 40. Calculating the capture efficiency as the ratio between particle counts of pre-control (or post-control) and sample, these capture efficiencies can be plotted versus voltage. The data fits an exponential association function with a high degree of Goodness of fit (0.983).

GLOSSARY

In the context of the present invention, the expressions such as "unit", "device" and "system" for example, as well as "detecting", "monitoring", "analyzing", "examining" and the like, may also be used interchangeably. The same applies for any other mutually equivalent expressions, as apparent to a person skilled in the art.

In the context of the present invention, the expression bioparticles refers to particles containing or comprising living or non-living microorganisms or spores thereof.

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CLAIMS

1. A micro scaled device for collecting biological particles comprising;

an impact collector having an inlet opening providing an air flow capability between the air to be sampled and the impact collector, and an outlet opening providing an air flow capability between the impact collector and the exterior of the impact collector, the outlet or inlet being connected to an air-flow producing means for drawing the air sample through the impact collector from the inlet opening to the outlet opening; said impact collector having a collecting component arranged within the impact collector between the inlet opening and the outlet opening, said collecting component consisting of two or more electrodes positioned in parallel and having the surfaces or at least a part of the surfaces coated with or consisting of material capable of leading an electrical current.

2. The method of claim 1, wherein said parallel electrodes enable the generation of an electrical field at an angle or perpendicular to the air-flow passing through the device, facilitating particles present in the sampled air to become charged and thereby being captured by adhering to either the positively or negatively charged electrode.

3. The method of claims 1 and 2, wherein said electrodes generate an electrical field of a size between 100 V/mm and 1500 V/mm.

4. The method of claim 1, wherein the biological particles are rinsed of the device using a rinsing fluid and means for drawing the fluid carrying the biological particles through the micro scaled device.

5. The method of claim 2, wherein the step of collecting the fluid carrying the biological particles comprises the step of directing said fluid into a reservoir, such that said fluid can be examined for the presence of biological particles.

FIGURES

FIGURE 1

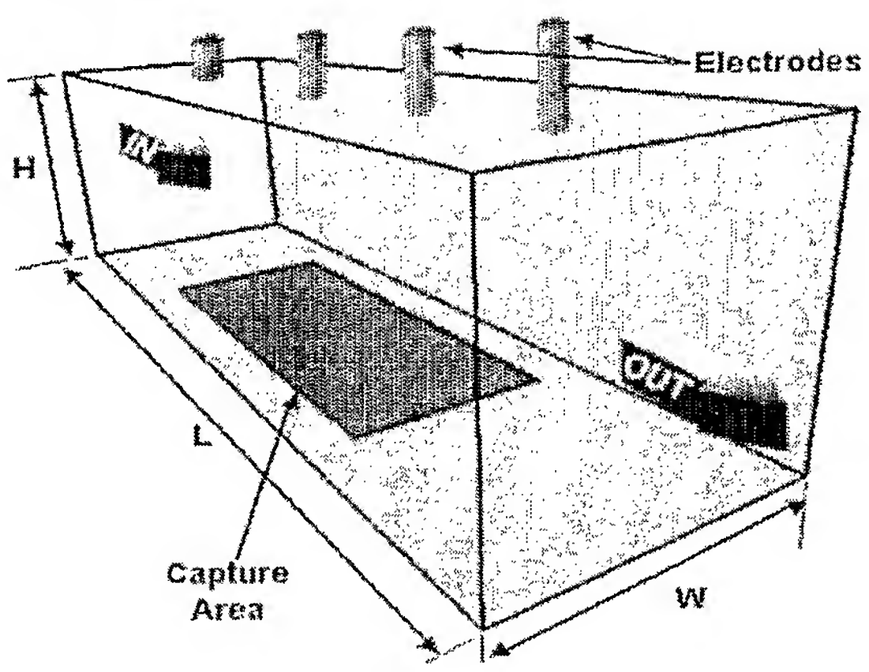

FIGURE 2

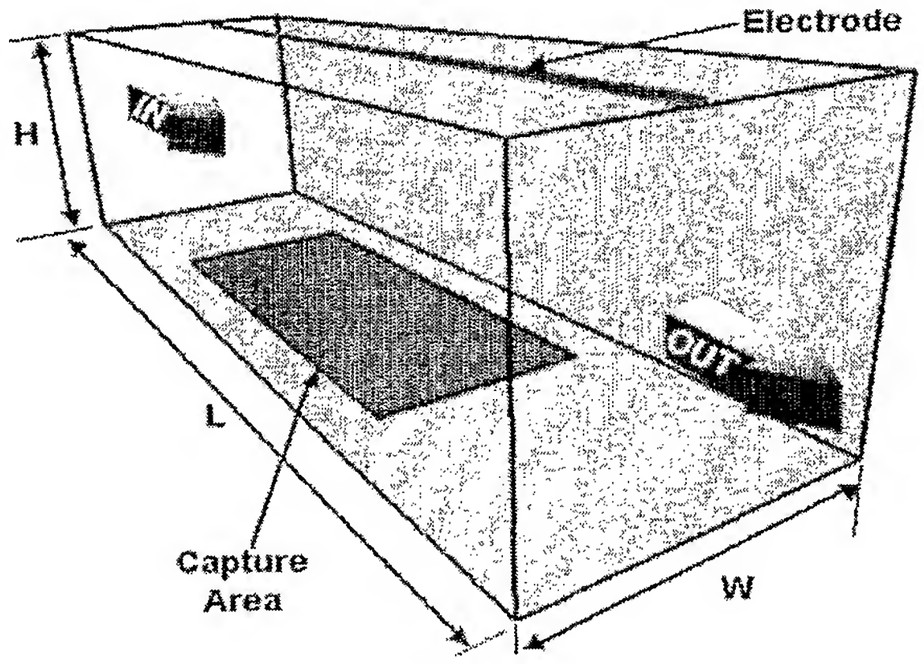

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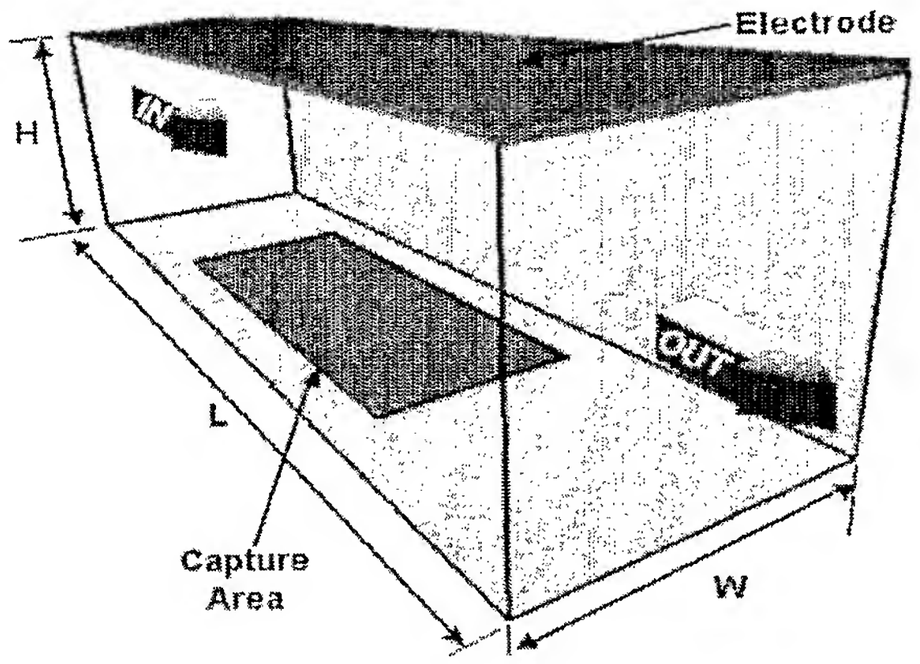
FIGURE 3



5 FIGURE 4

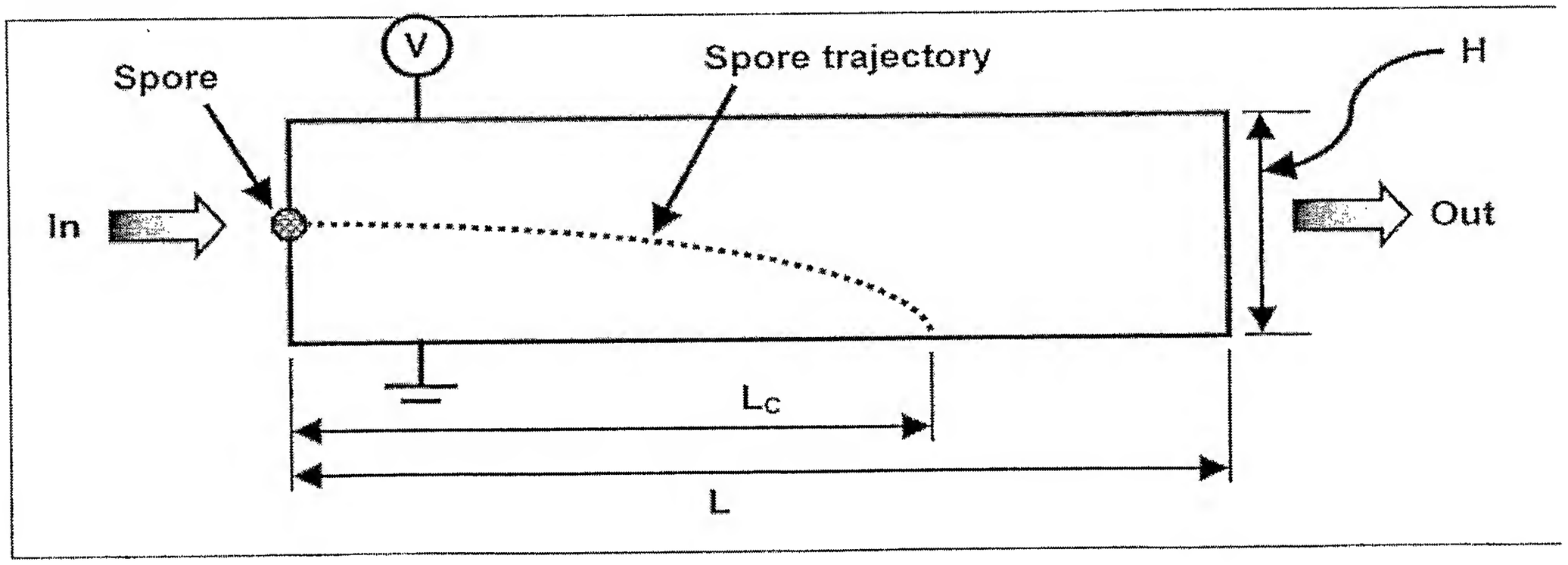


FIGURE 5

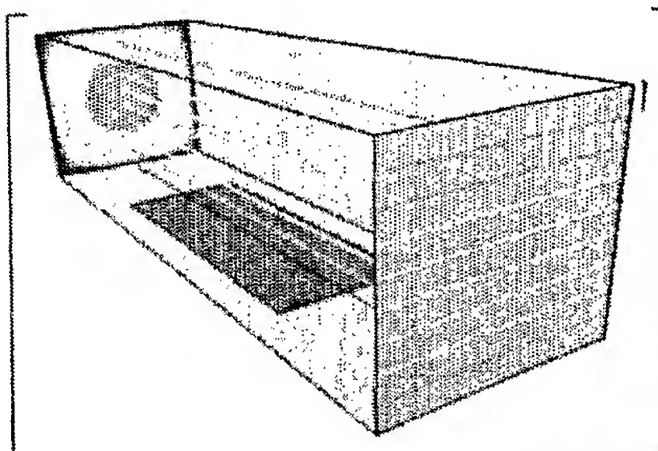


Plate Electrode	Wire Electrode	Point Electrode (10 wires, 250 µm apart)

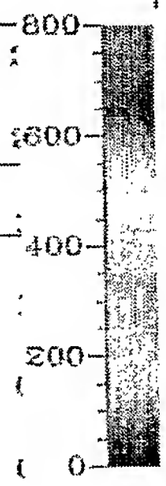


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FIGURE 6

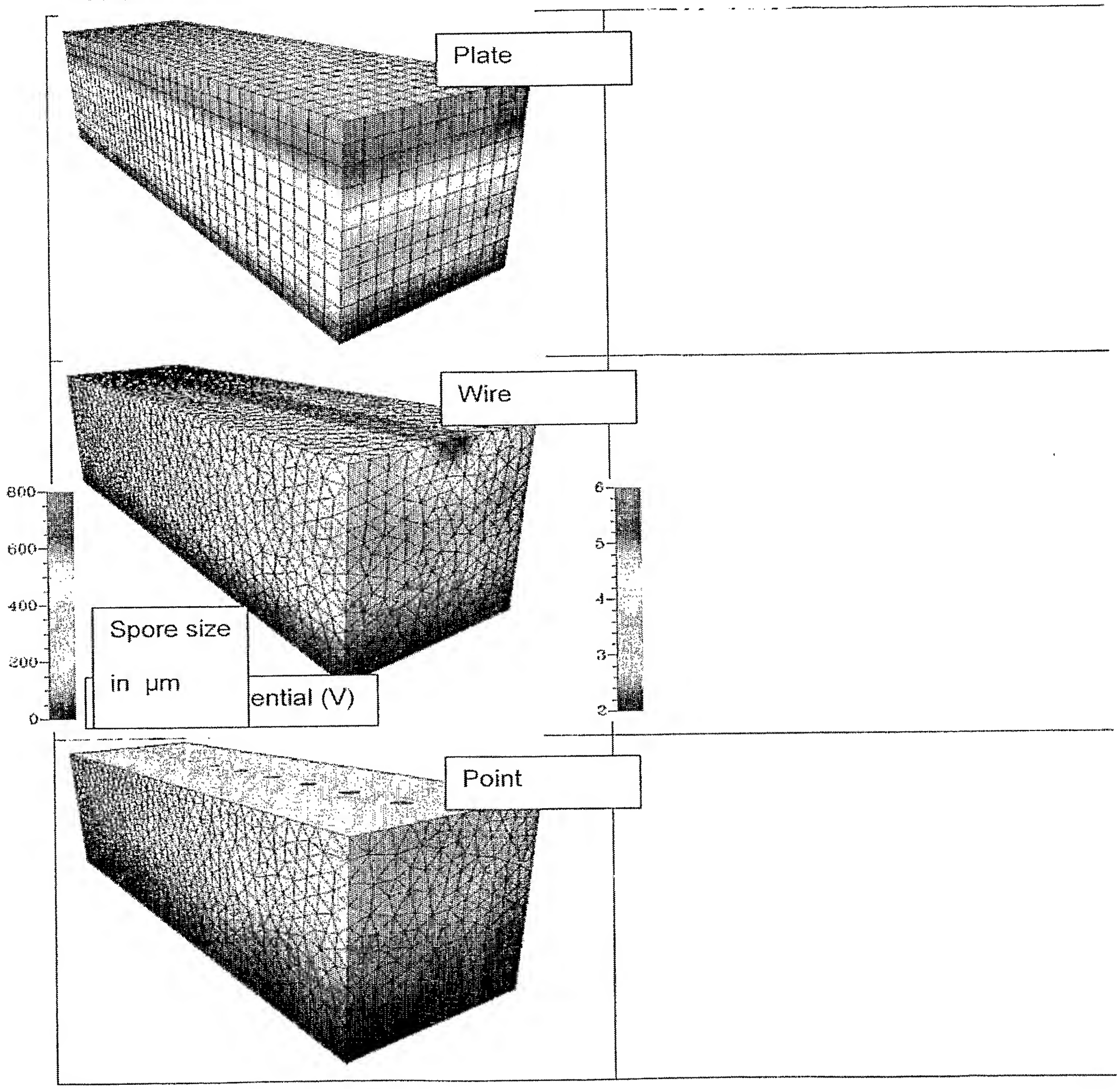

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FIGURE 7a, plate design

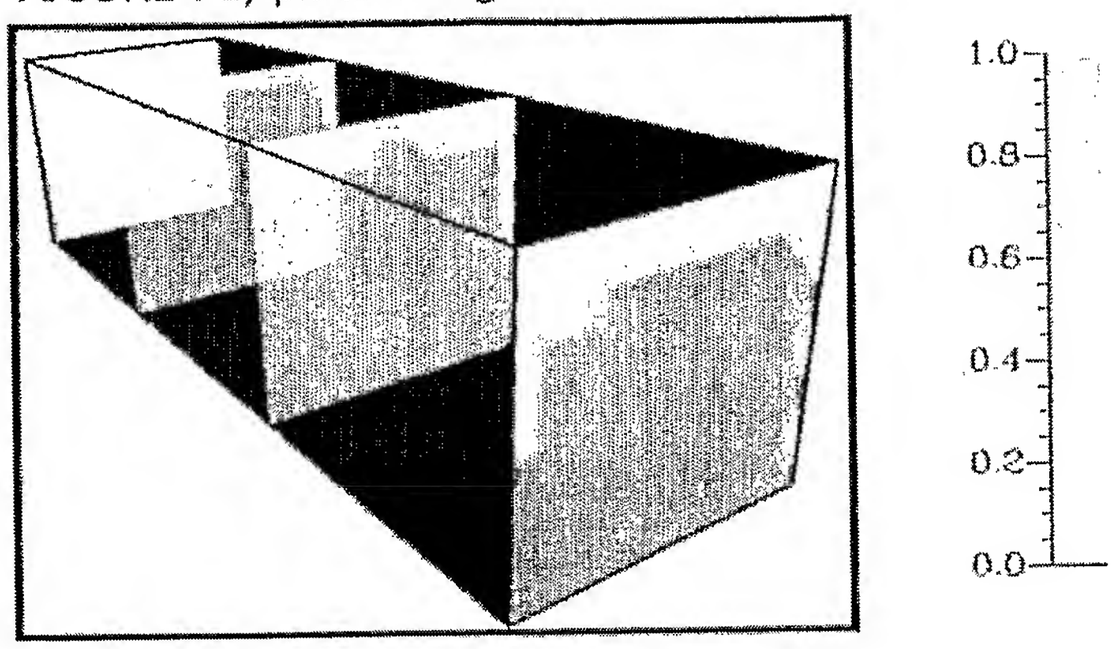

FIGURE 7b, wire design

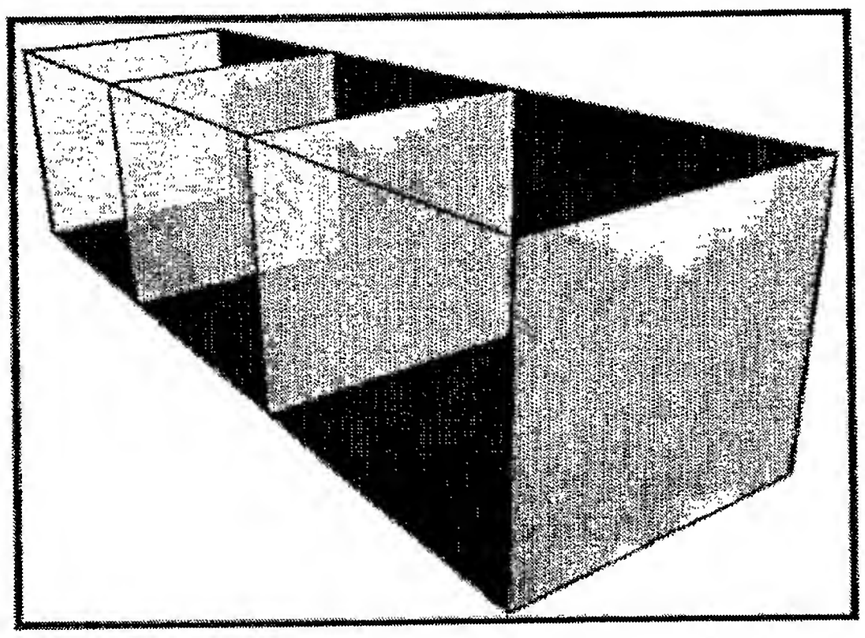

FIGURE 7c, point electrode design

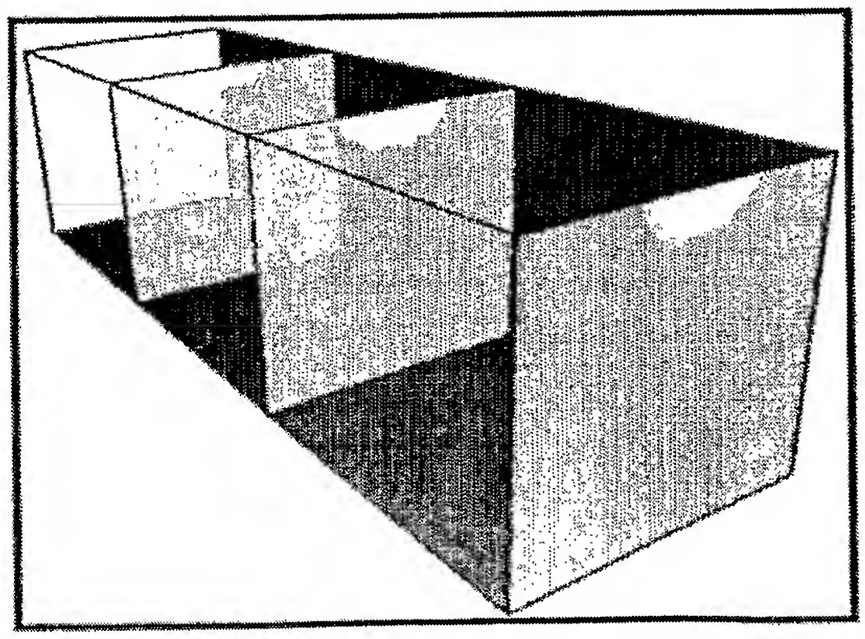

FIGURE 8a

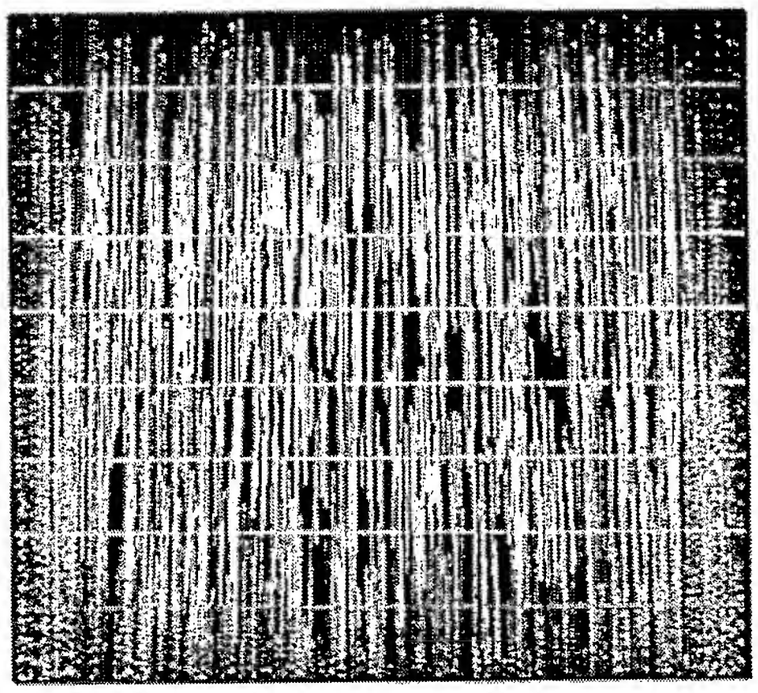

FIGURE 8b

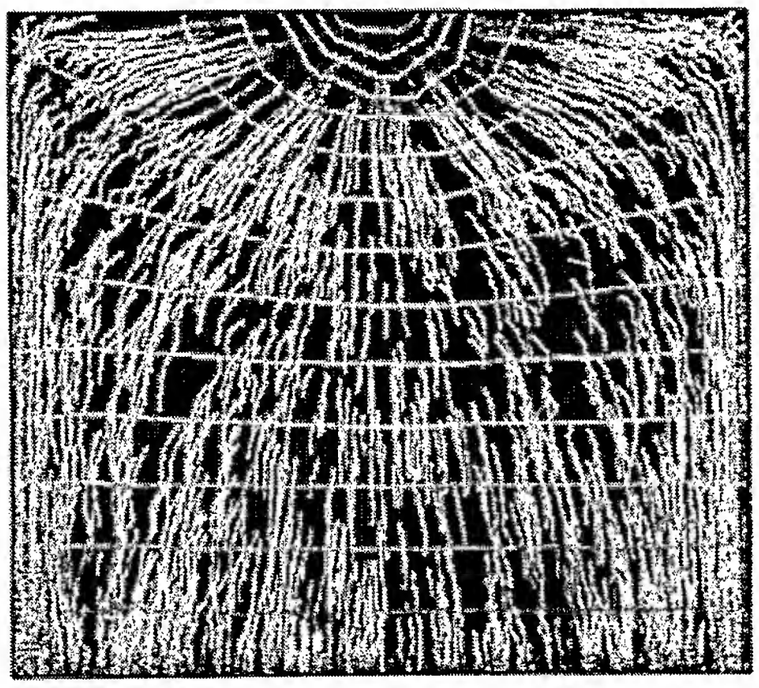

FIGURE 8c

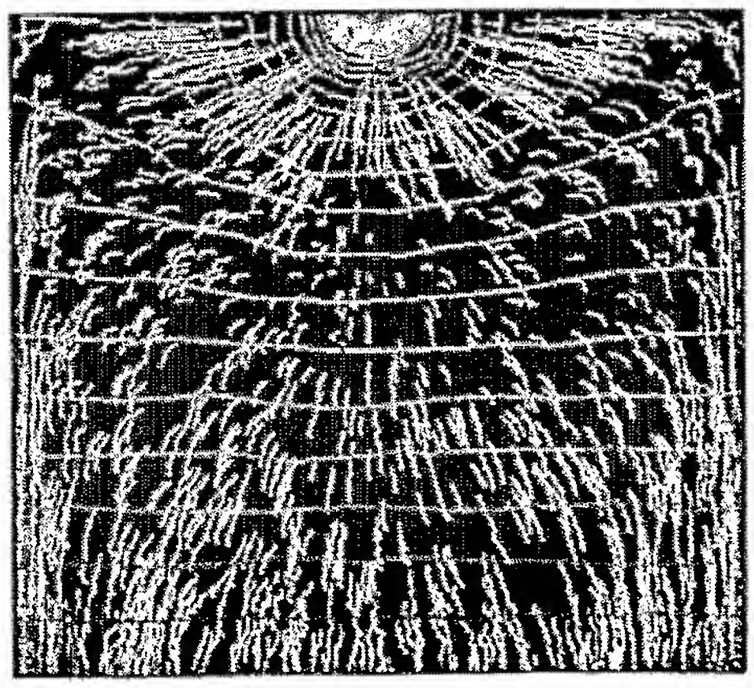

FIGURE 9

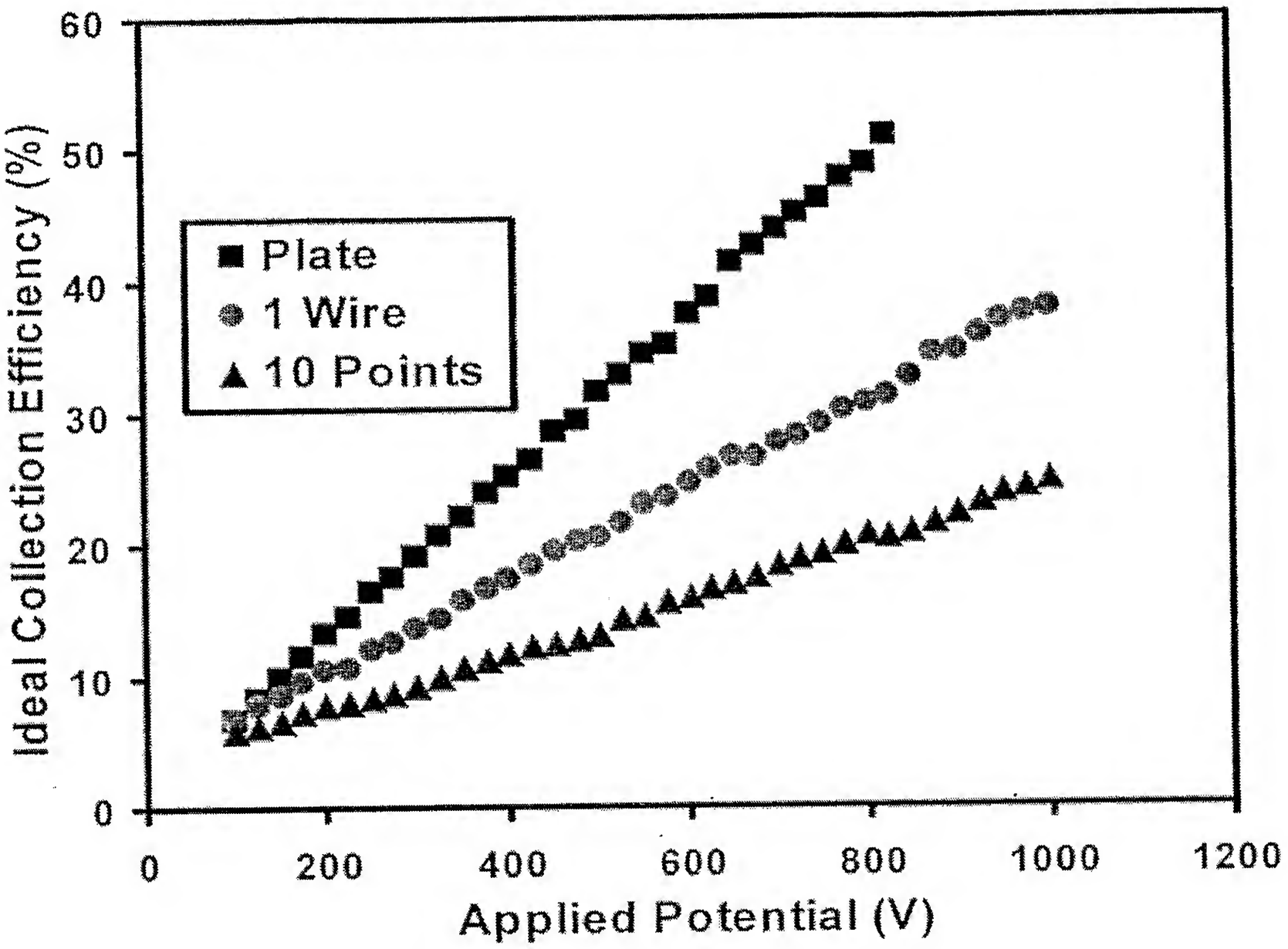

FIGURE 10

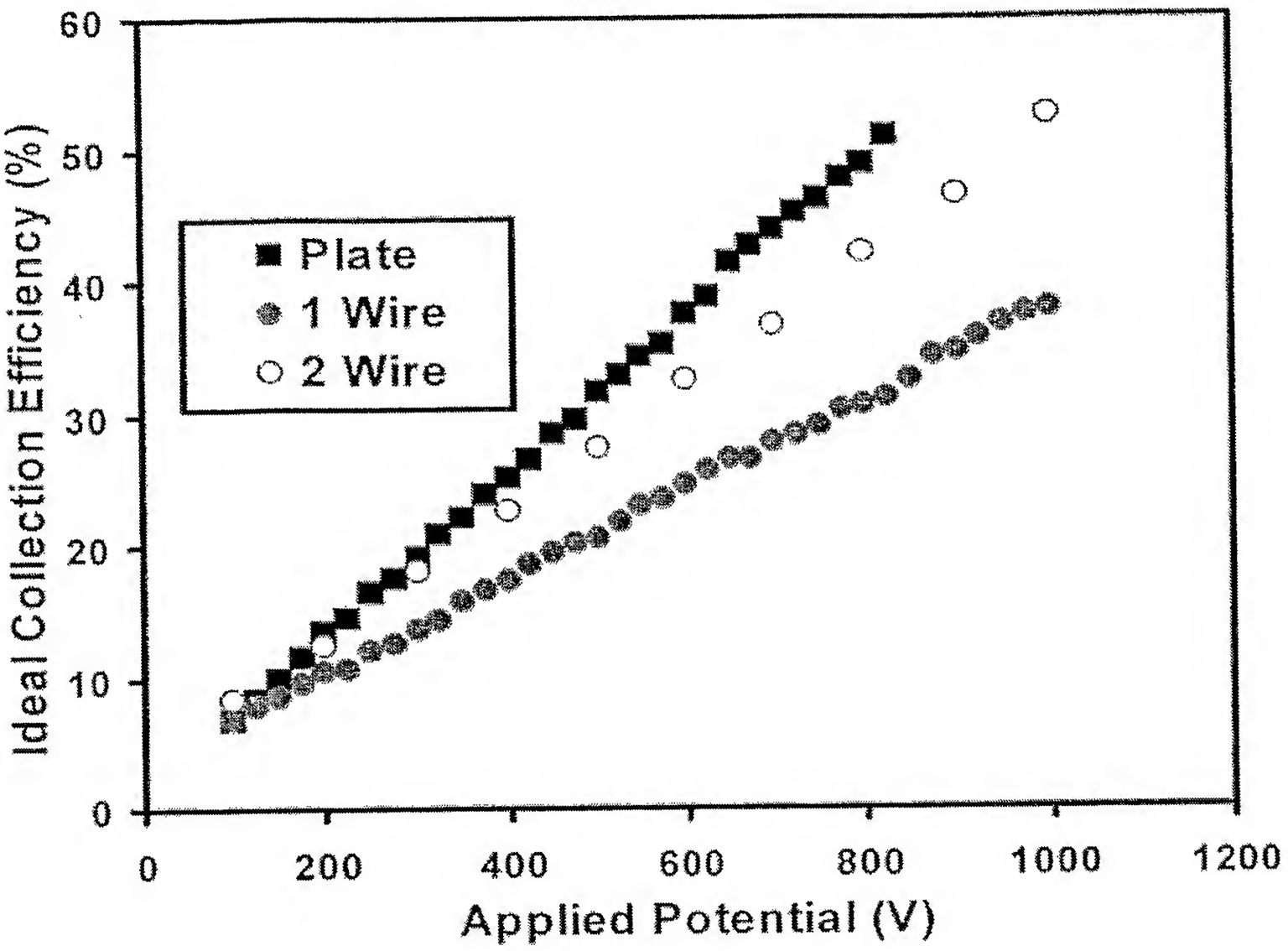

FIGURE 11

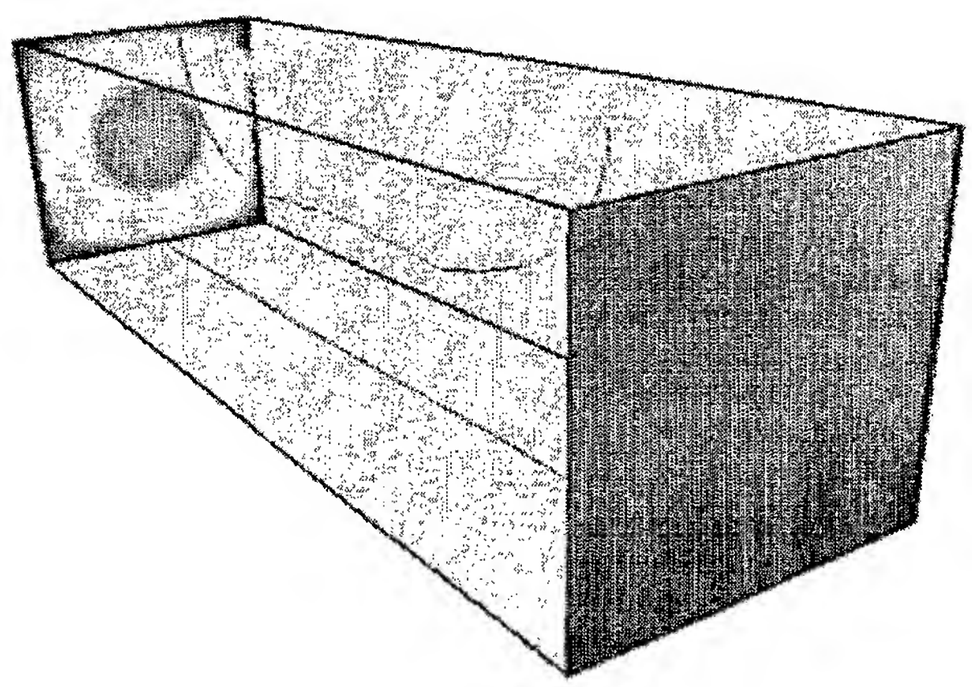

FIGURE 12

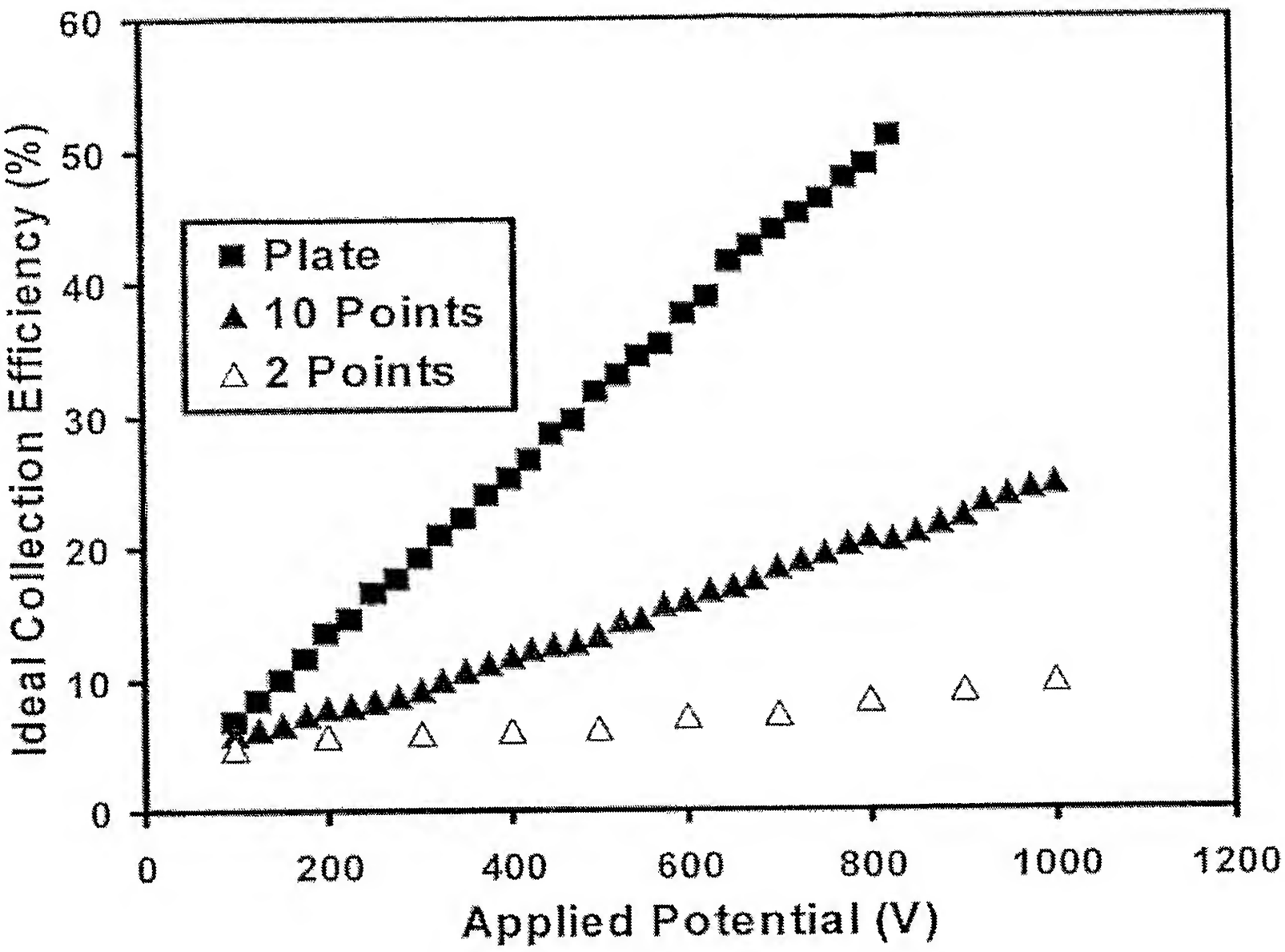

Figure 13

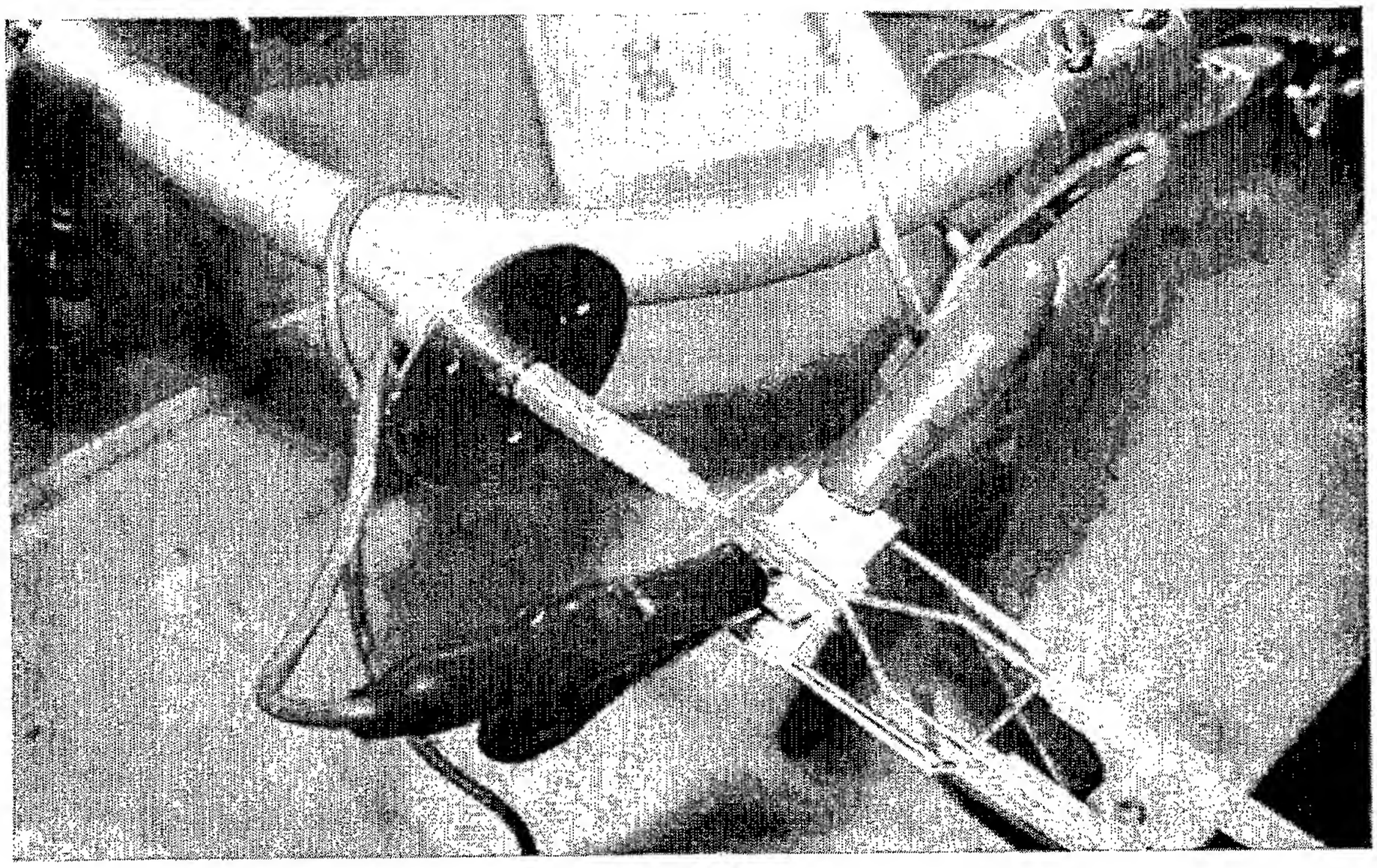

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Figure 14

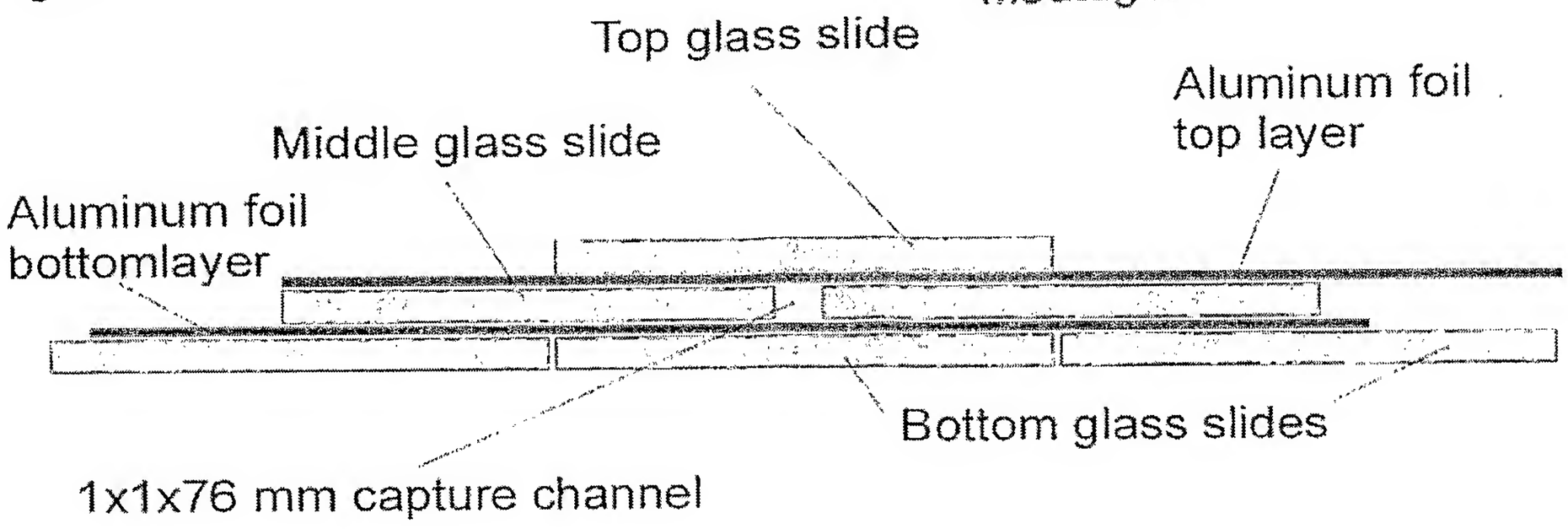

Figure 15a

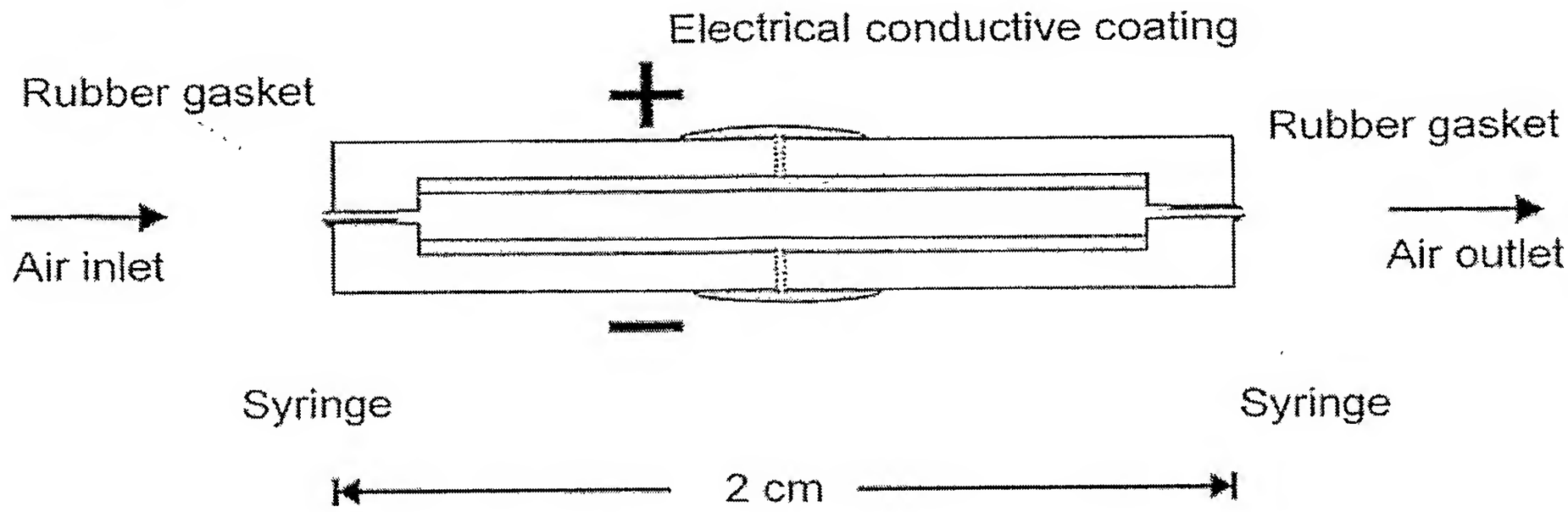

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Figure 15b

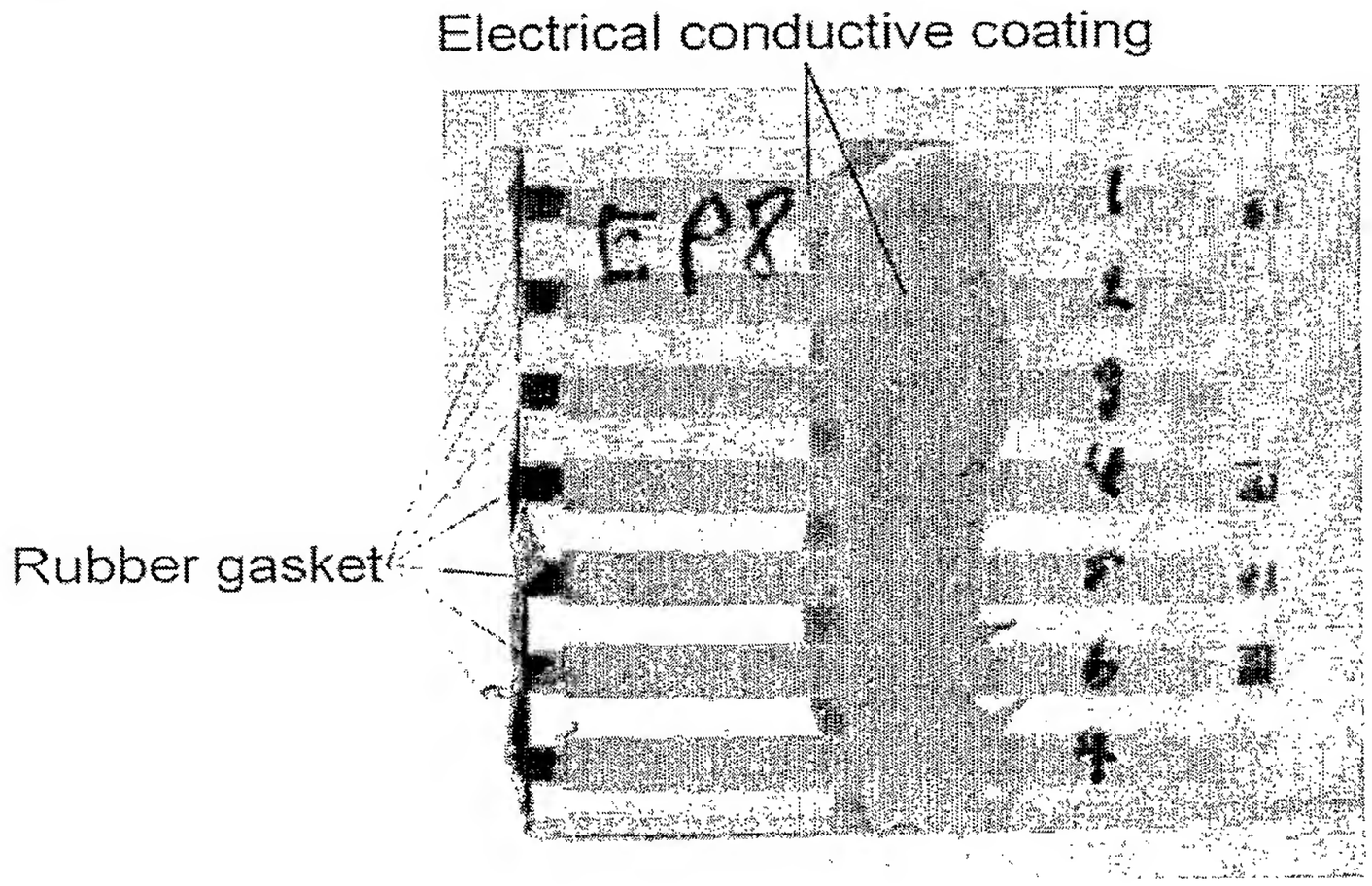

Figure 16. Chip collector A. Samples taken with 100 V difference over electrodes.

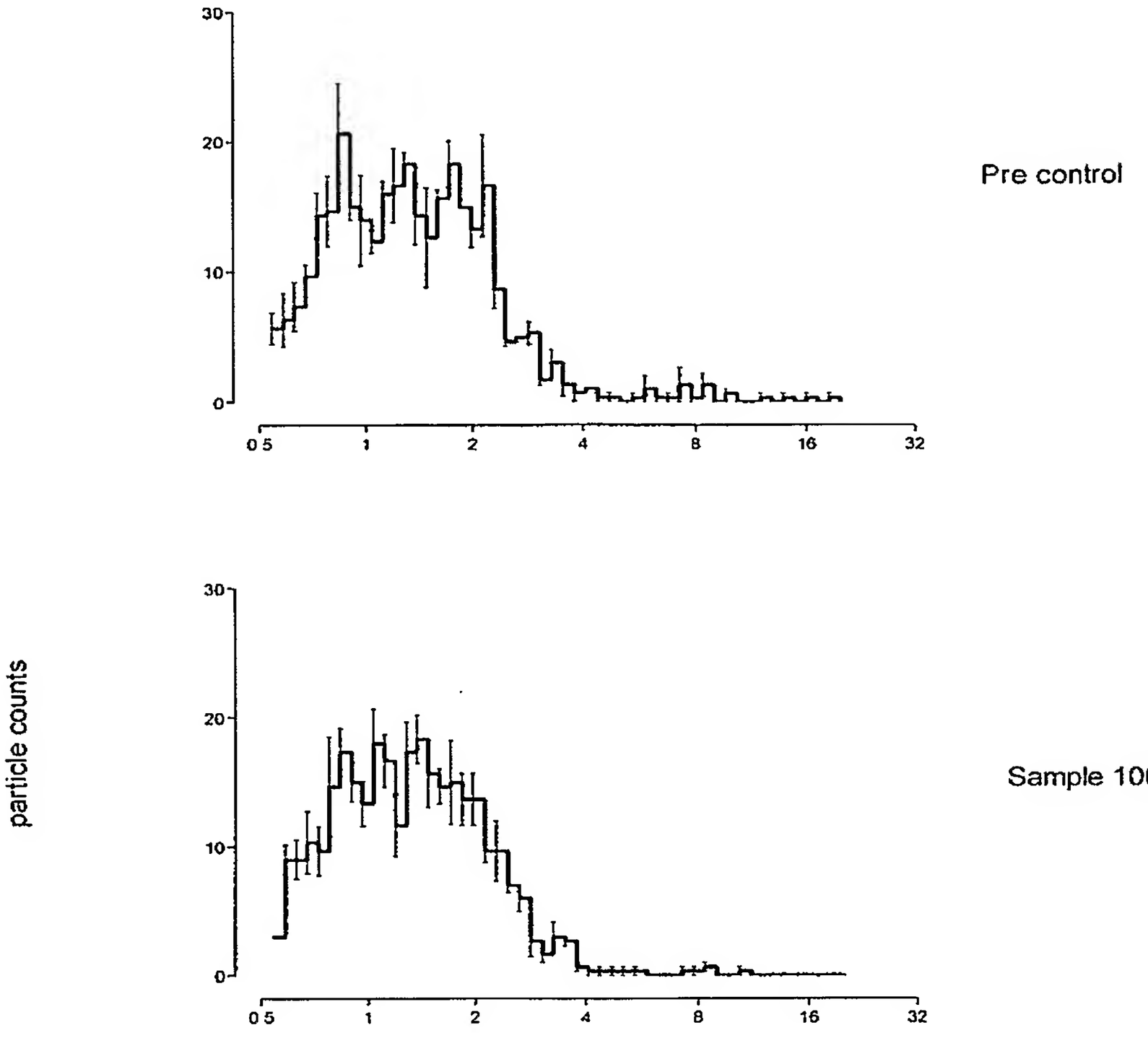

Figure 17. Chip collector A. Samples taken with 200 V difference over electrodes.

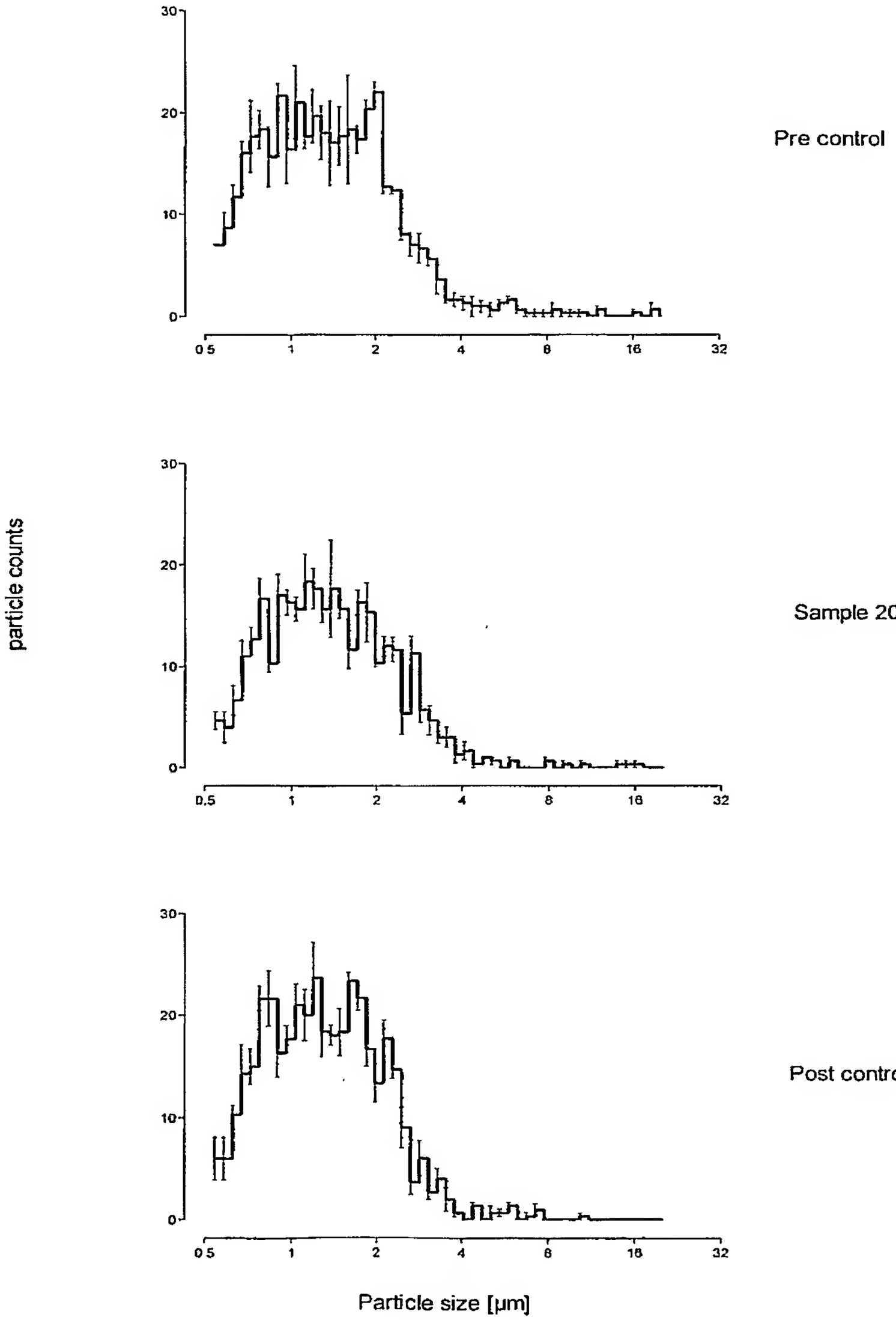

Figure 18. Chip collector A. Samples taken with 300 V difference over electrodes.

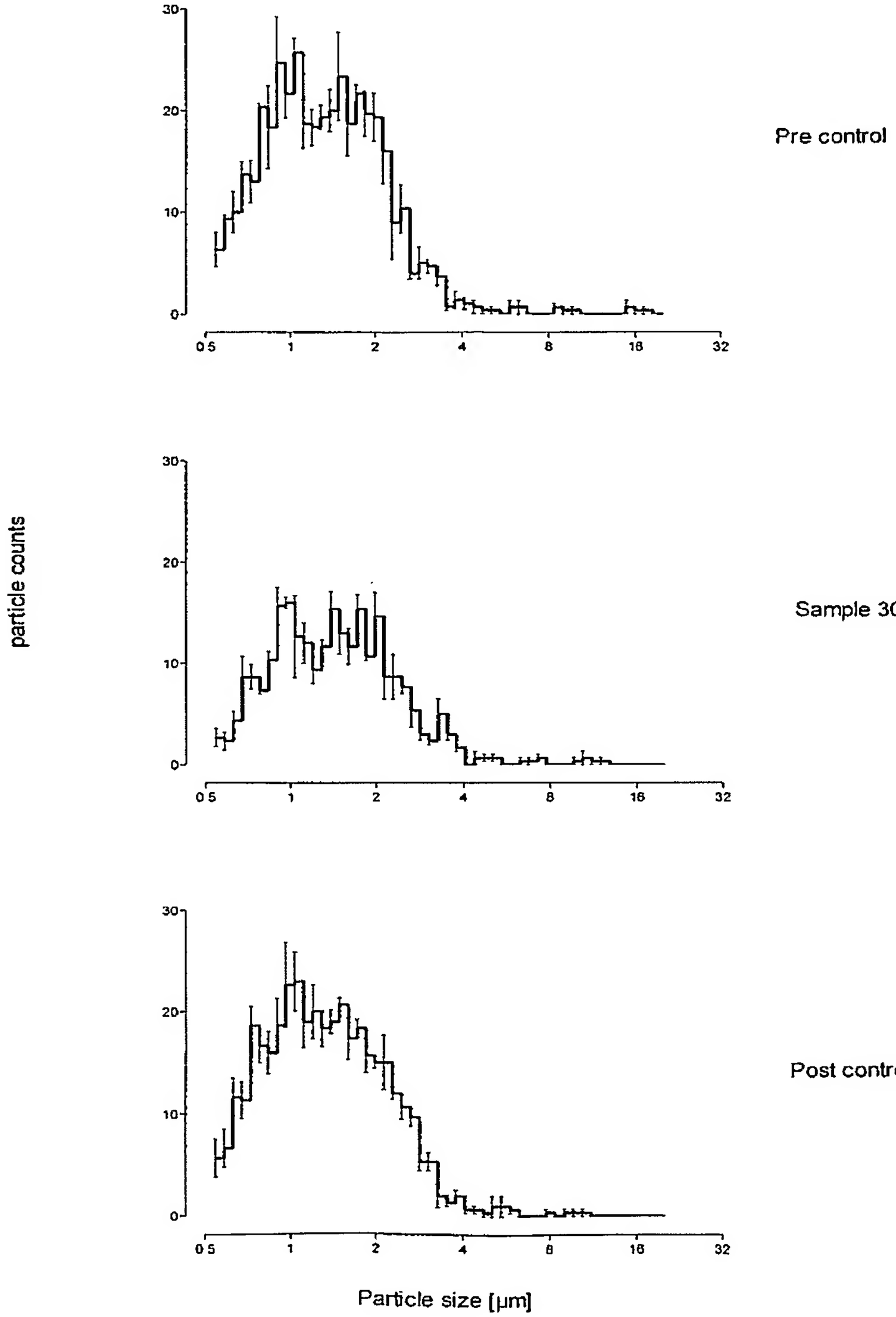

Figure 19. Chip collector A. Samples taken with 400 V difference over electrodes.

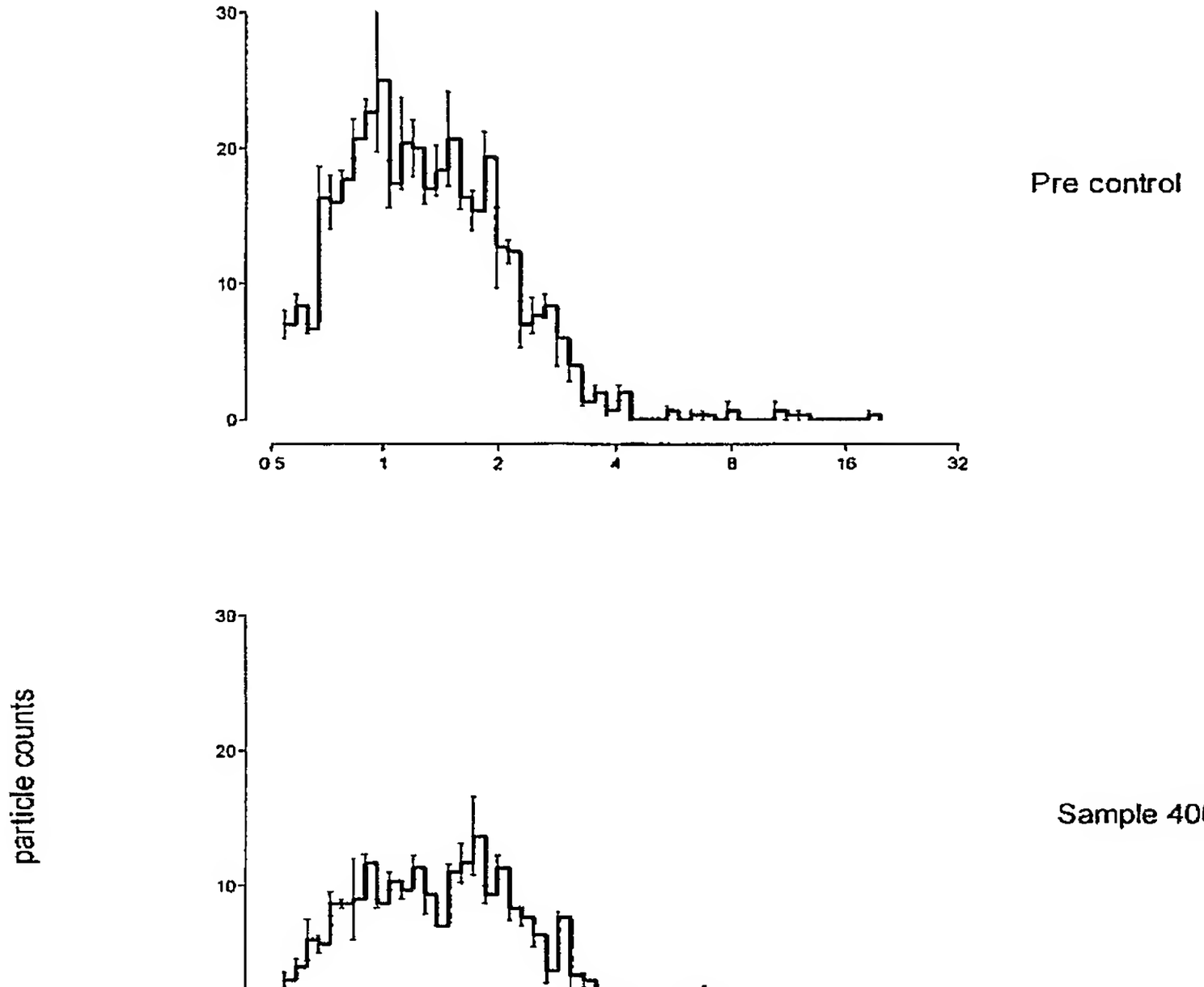

Figure 20. Chip collector A. Samples taken with 500 V difference over electrodes.

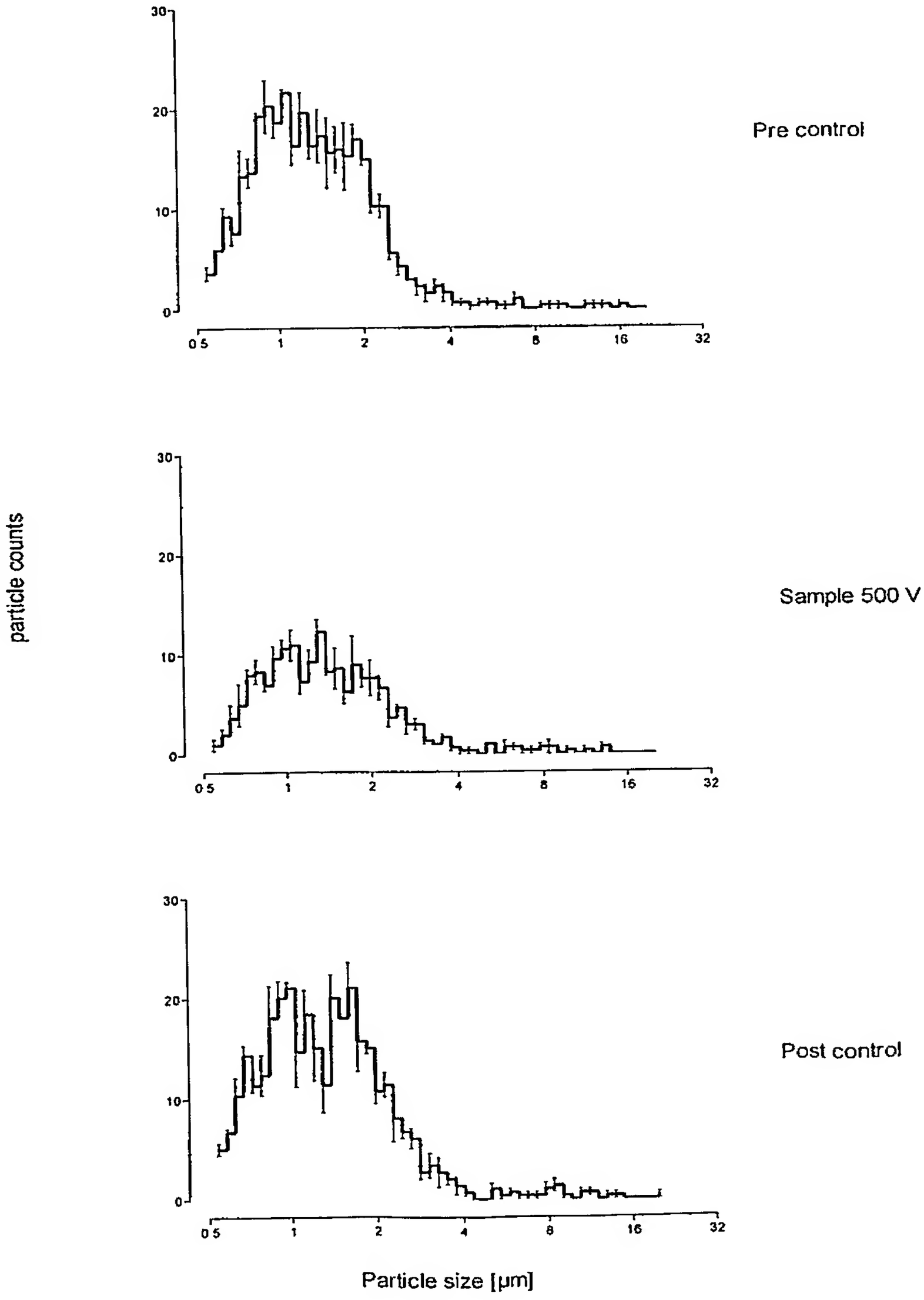

Figure 21. Chip collector A. Samples taken with 600 V difference over electrodes.

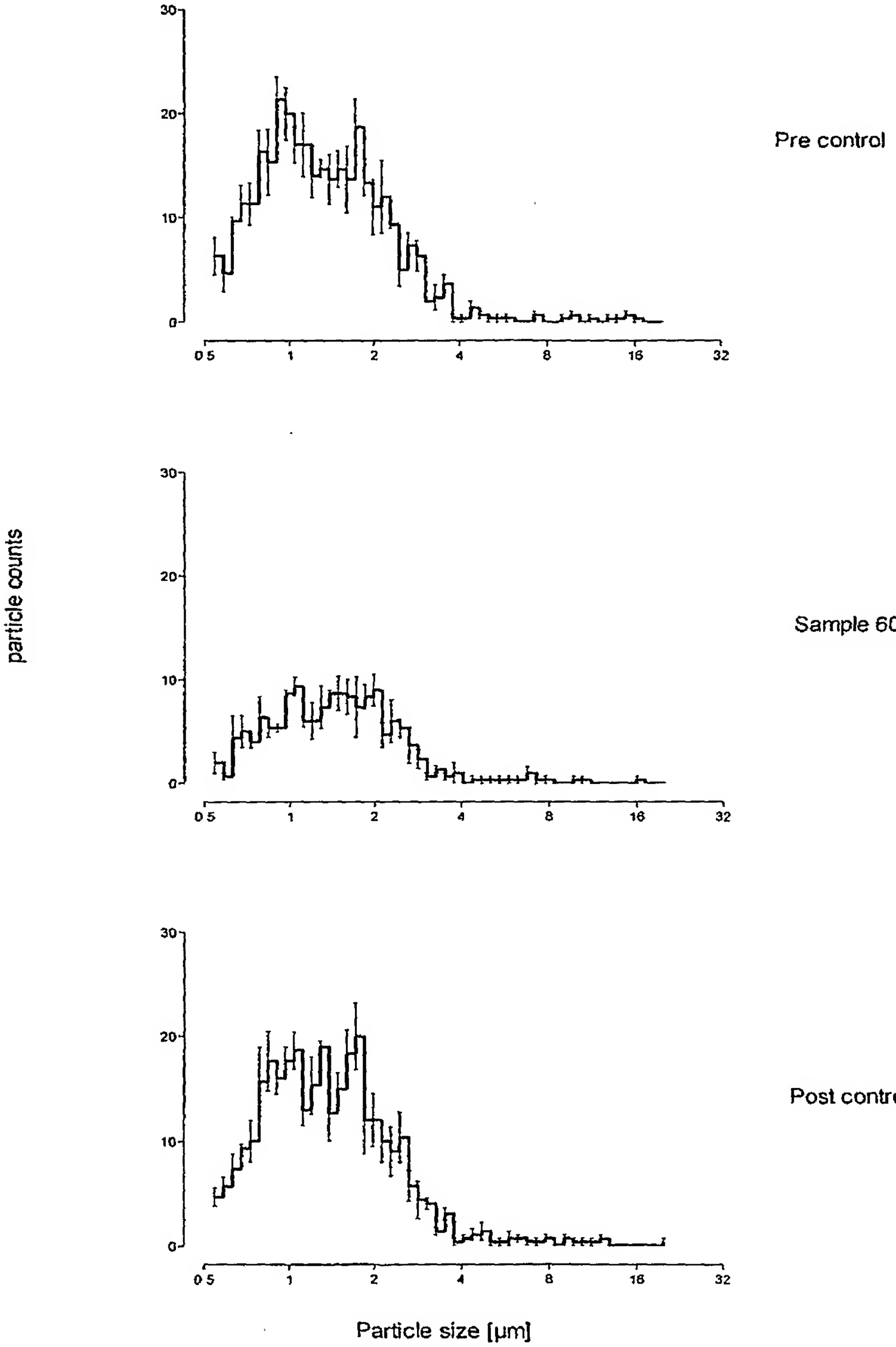

Figure 22. Chip collector A. Samples taken with 700 V difference over electrodes.

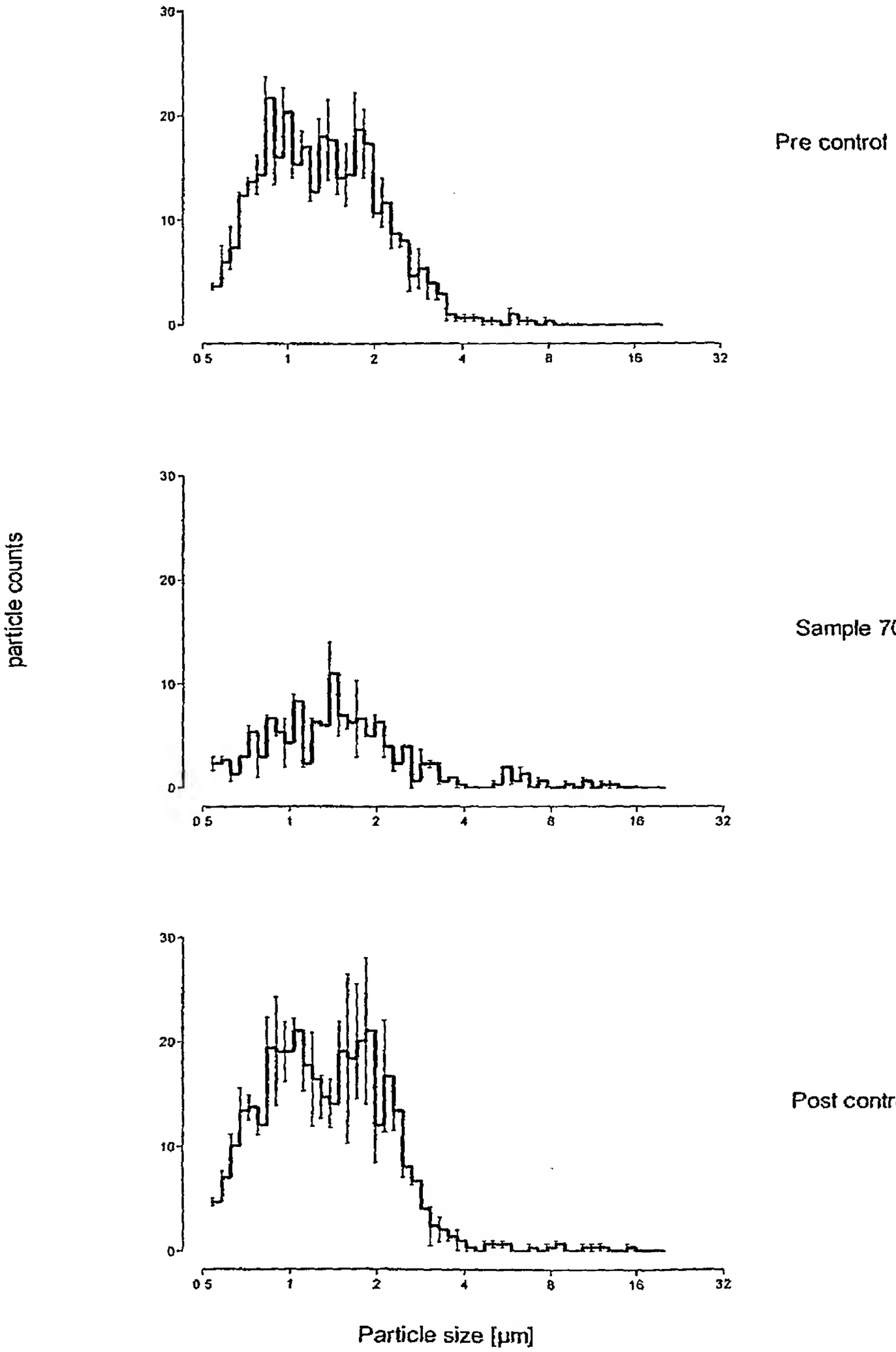

Figure 23. Chip collector A. Samples taken with 800 V difference over electrodes.

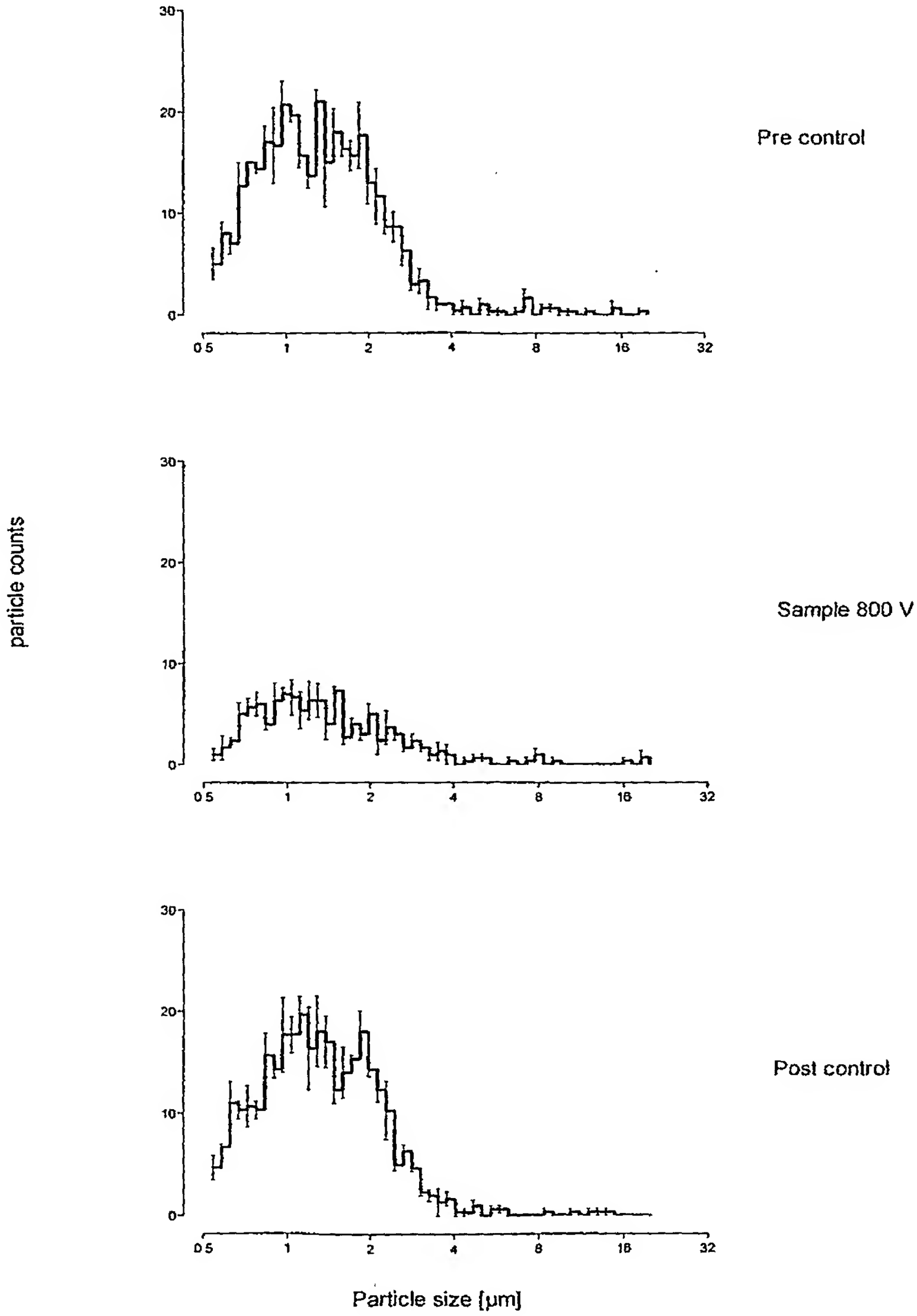

Figure 24. Chip collector A. Samples taken with 900 V difference over electrodes

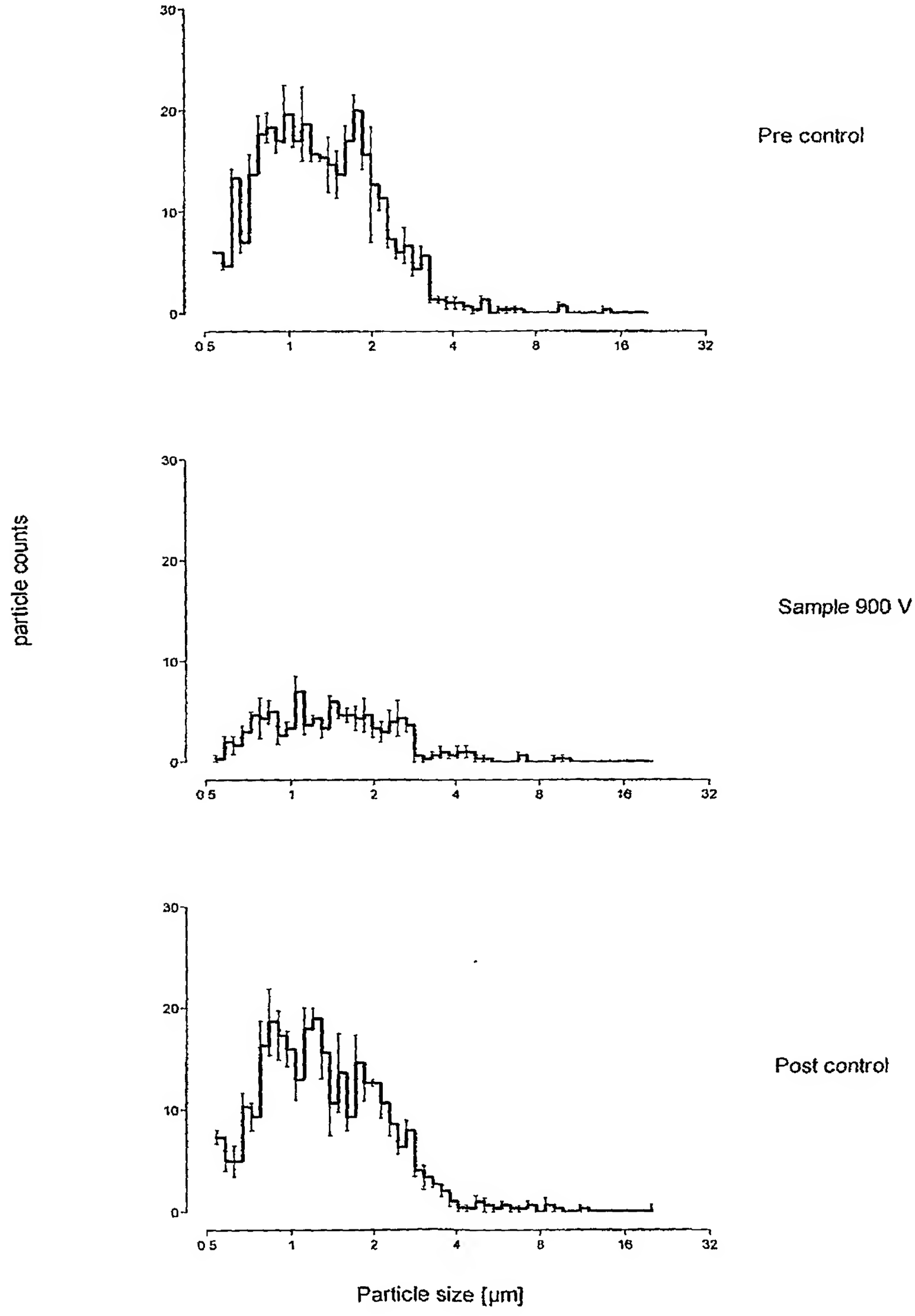

Figure 25. Chip collector A. Samples taken with 1000 V difference over electrodes.

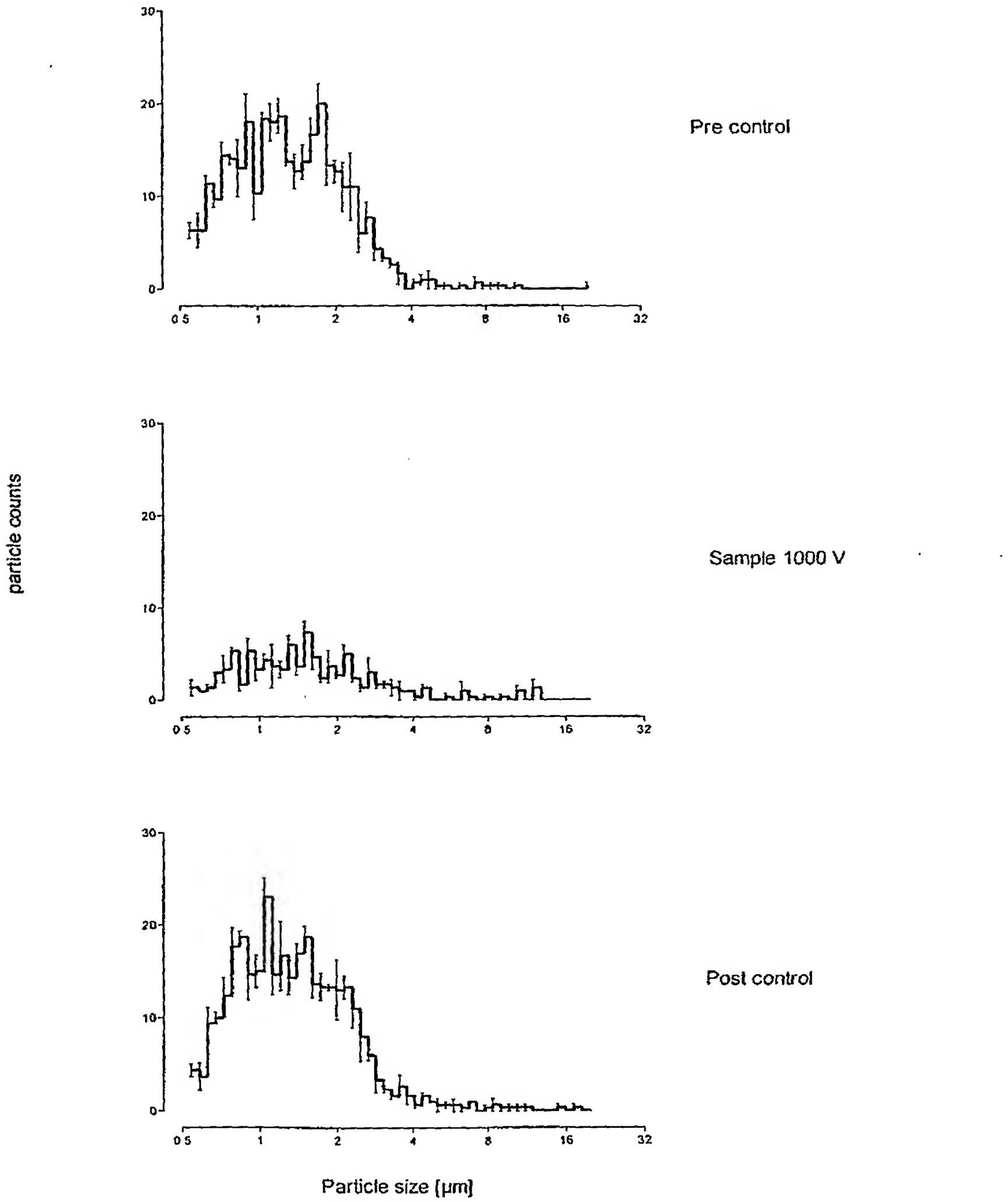

Figure 26. Chip collector A. Samples taken with 1100 V difference over electrodes.

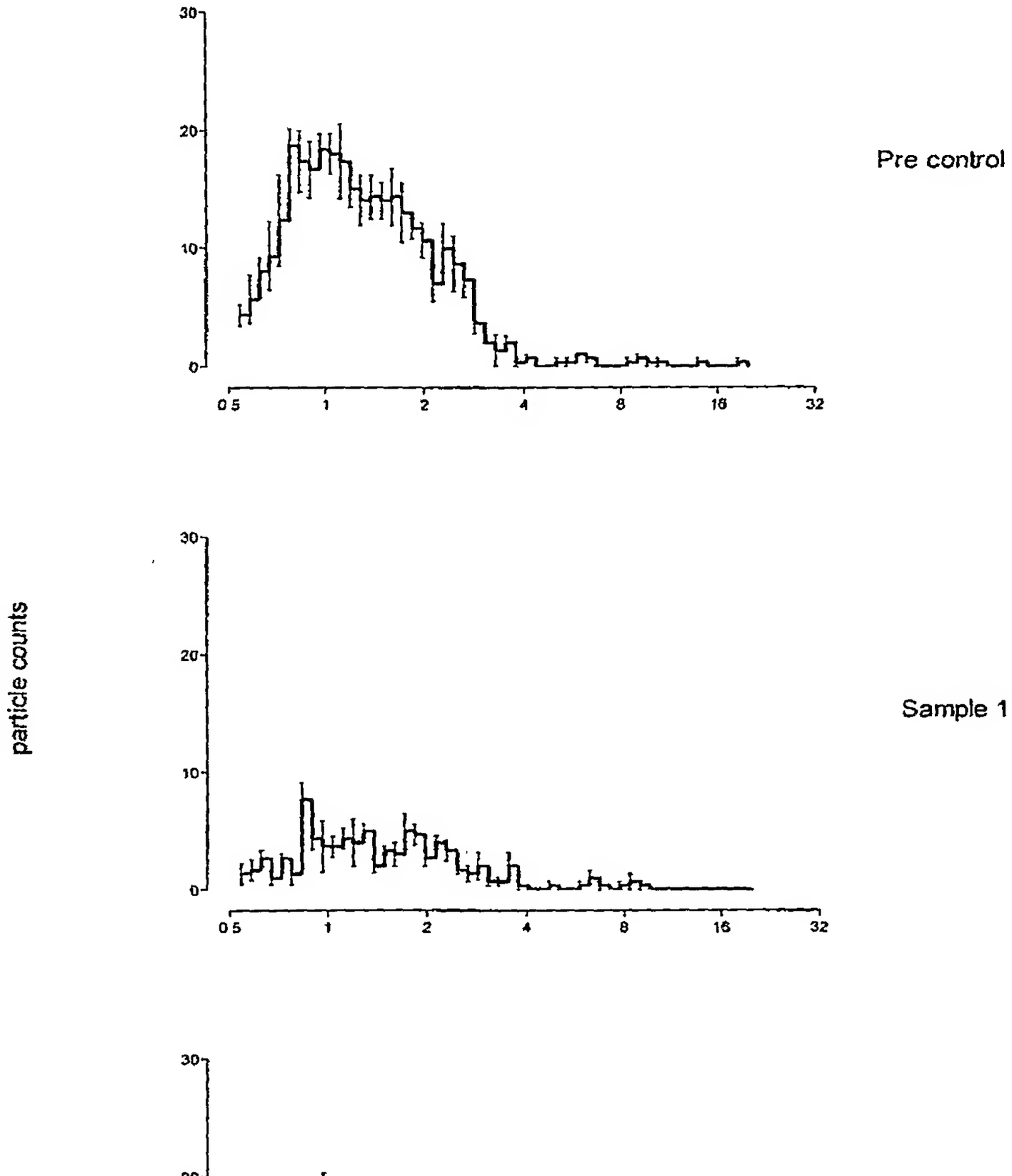

Figure 27. Chip collector A. Samples taken with 1200 V difference over electrodes.

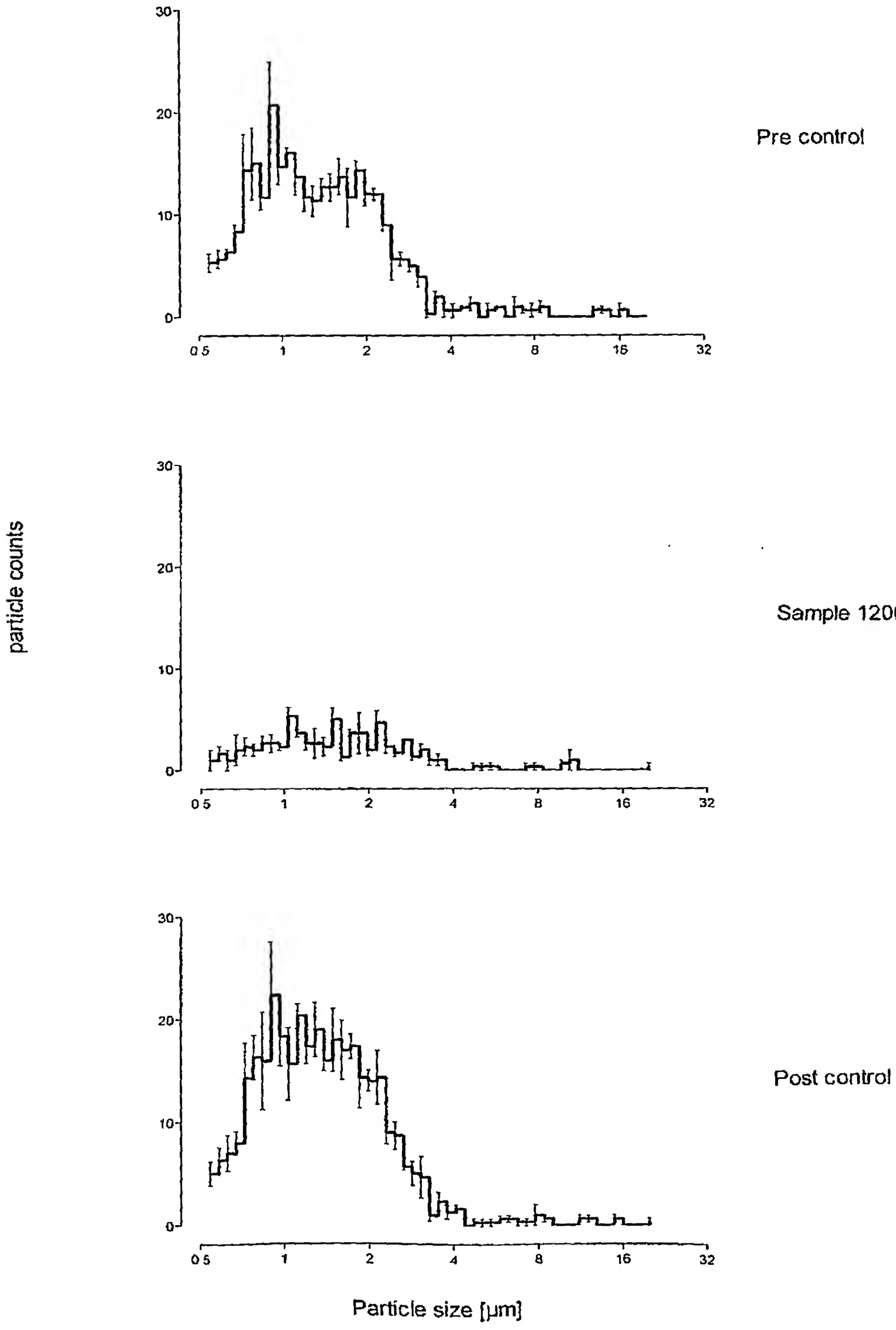

Figure 28. Chip collector B. Samples taken with 100 V difference over electrodes.

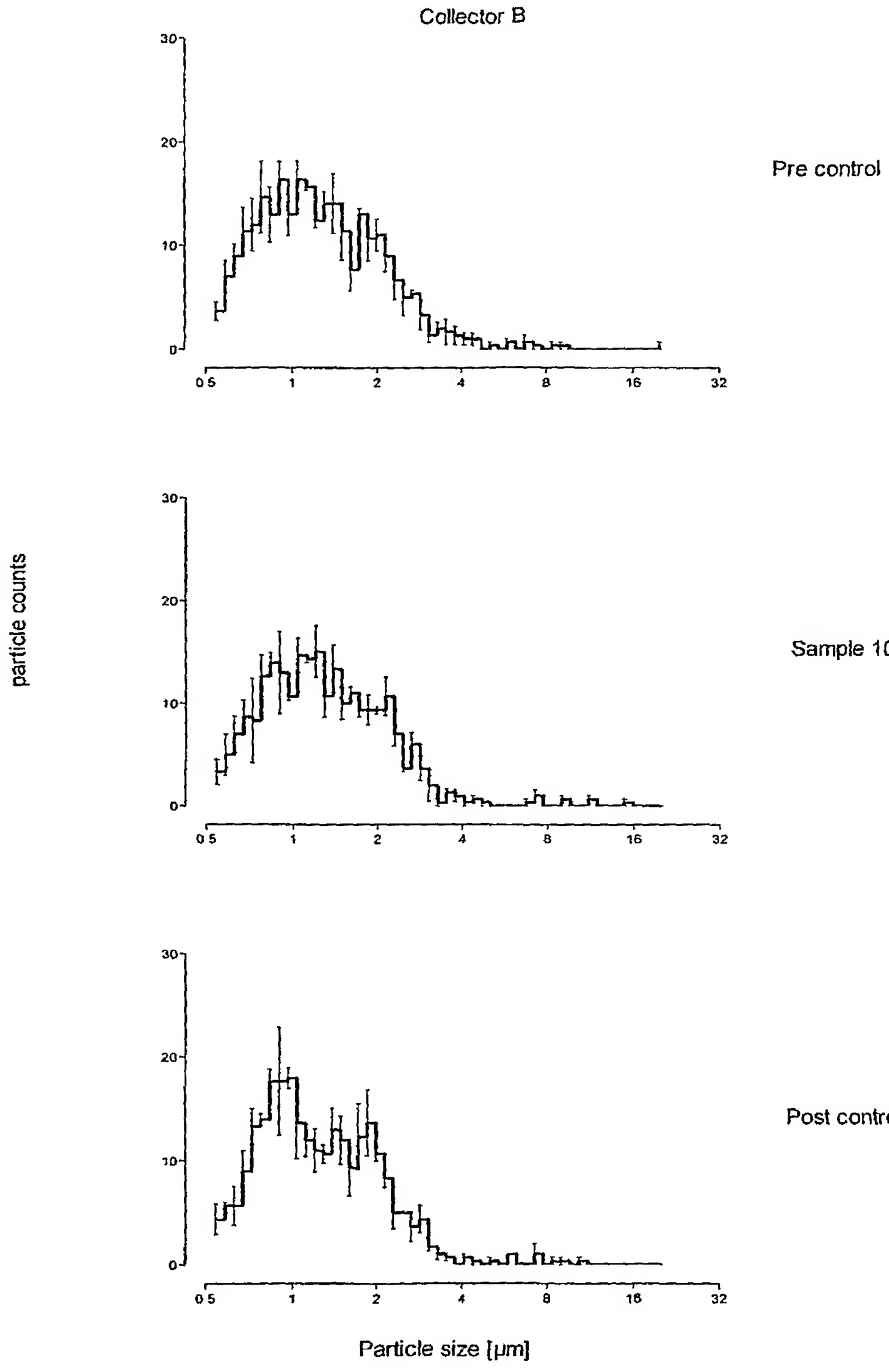

Figure 29. Chip collector B. Samples taken with 200 V difference over electrodes.

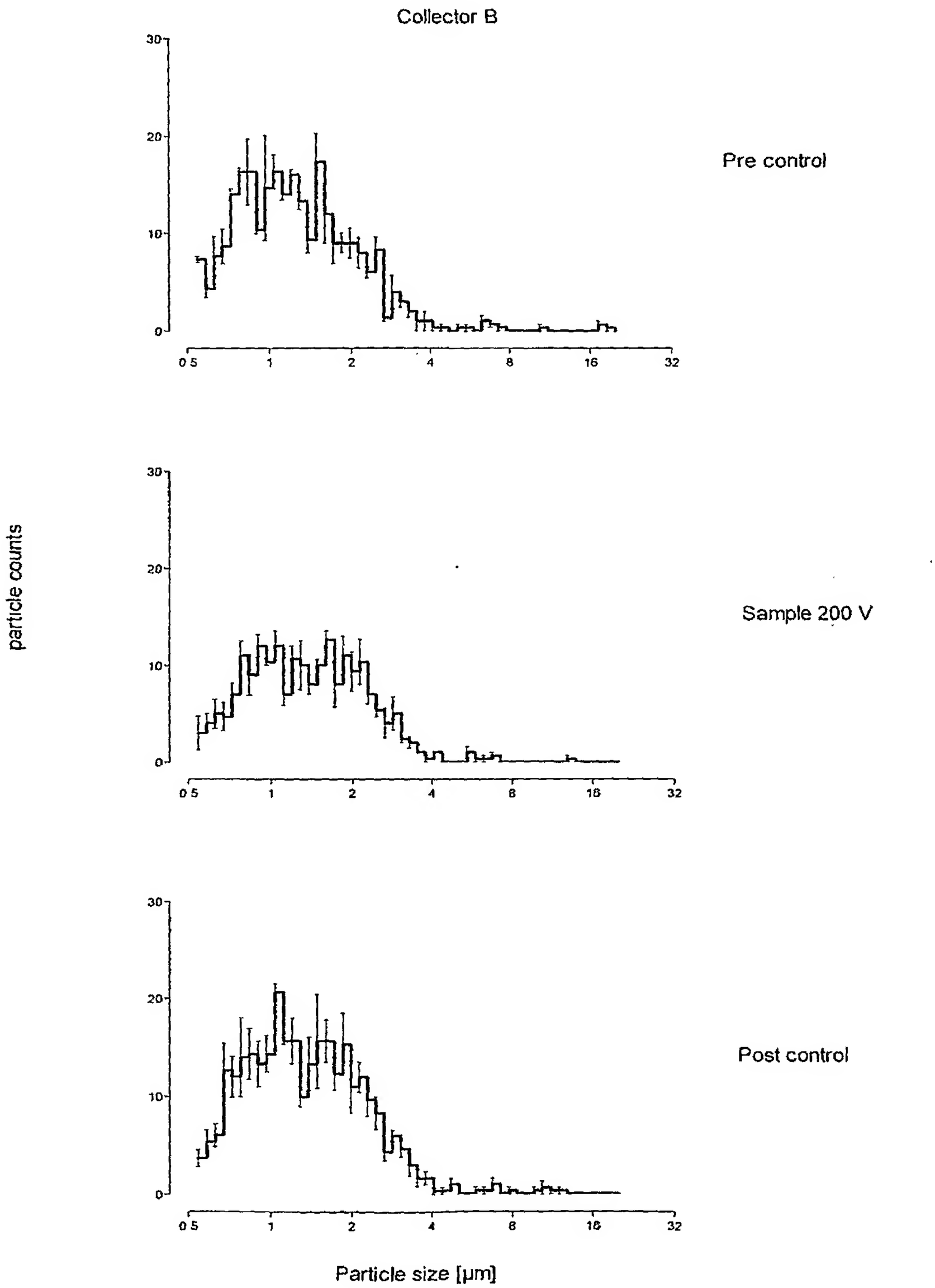

Figure 30. Chip collector B. Samples taken with 300 V difference over electrodes.

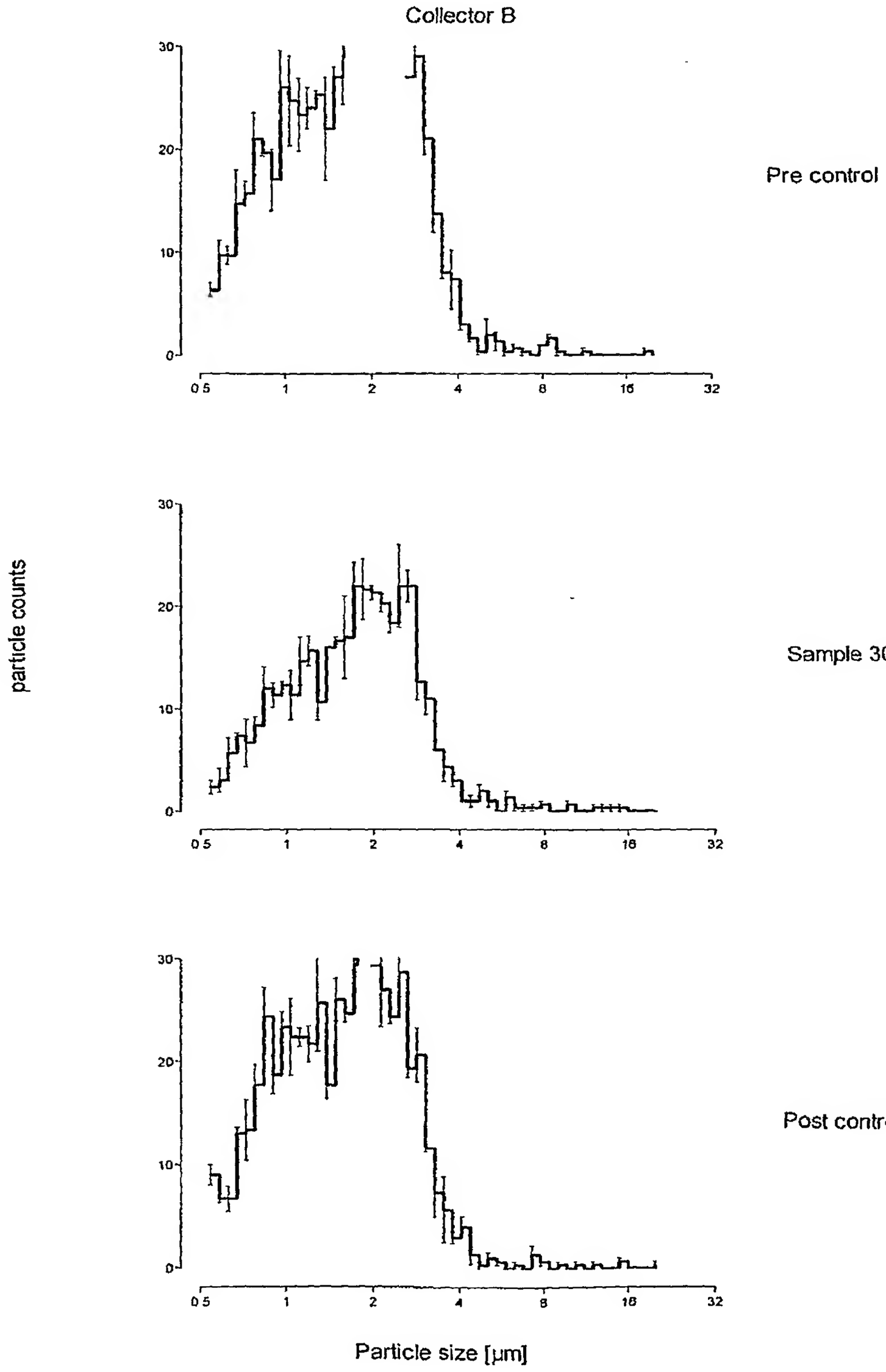

Figure 31. Chip collector B. Samples taken with 400 V difference over electrodes.

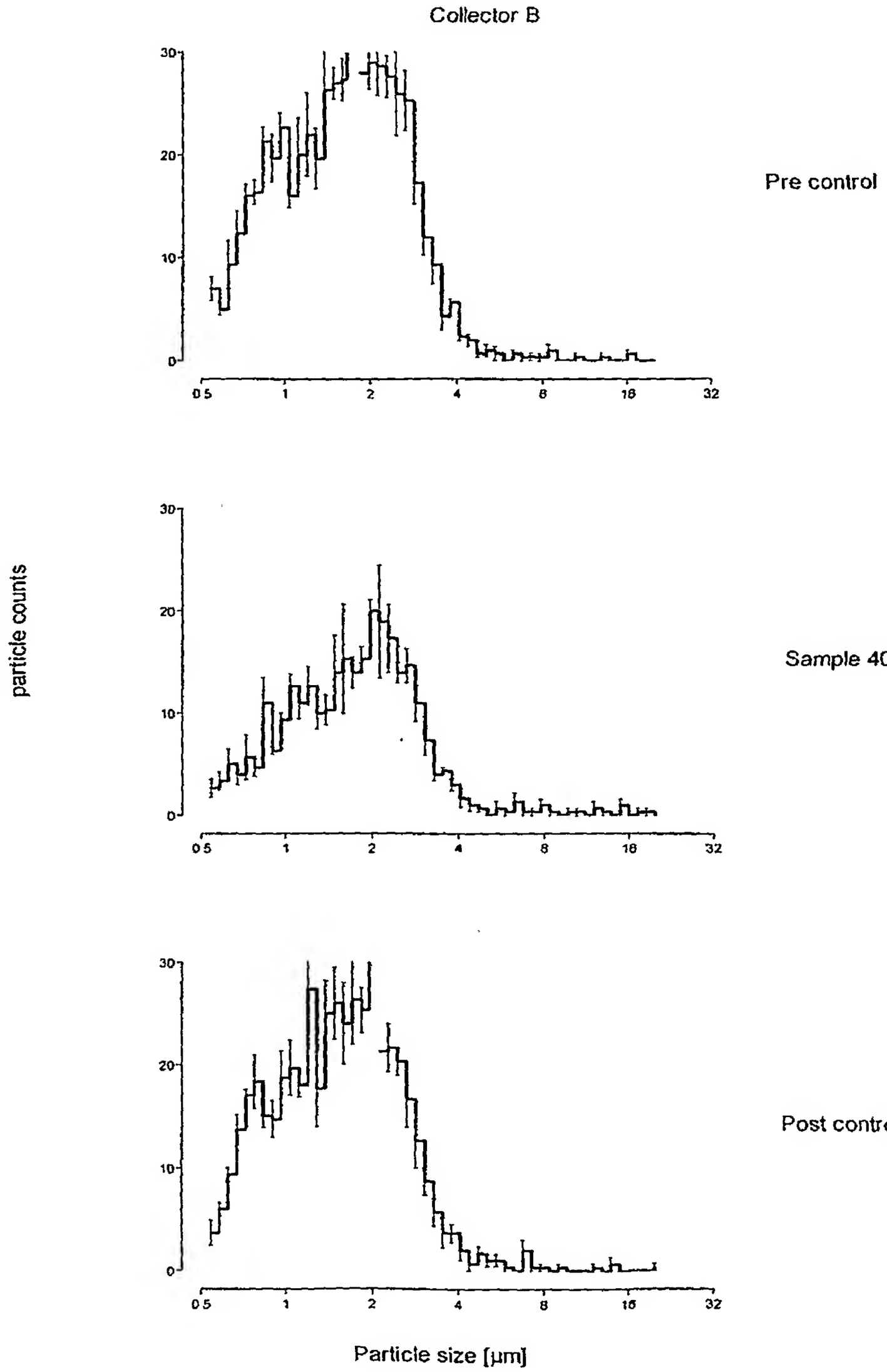

Figure 32. Chip collector B. Samples taken with 500 V difference over electrodes.

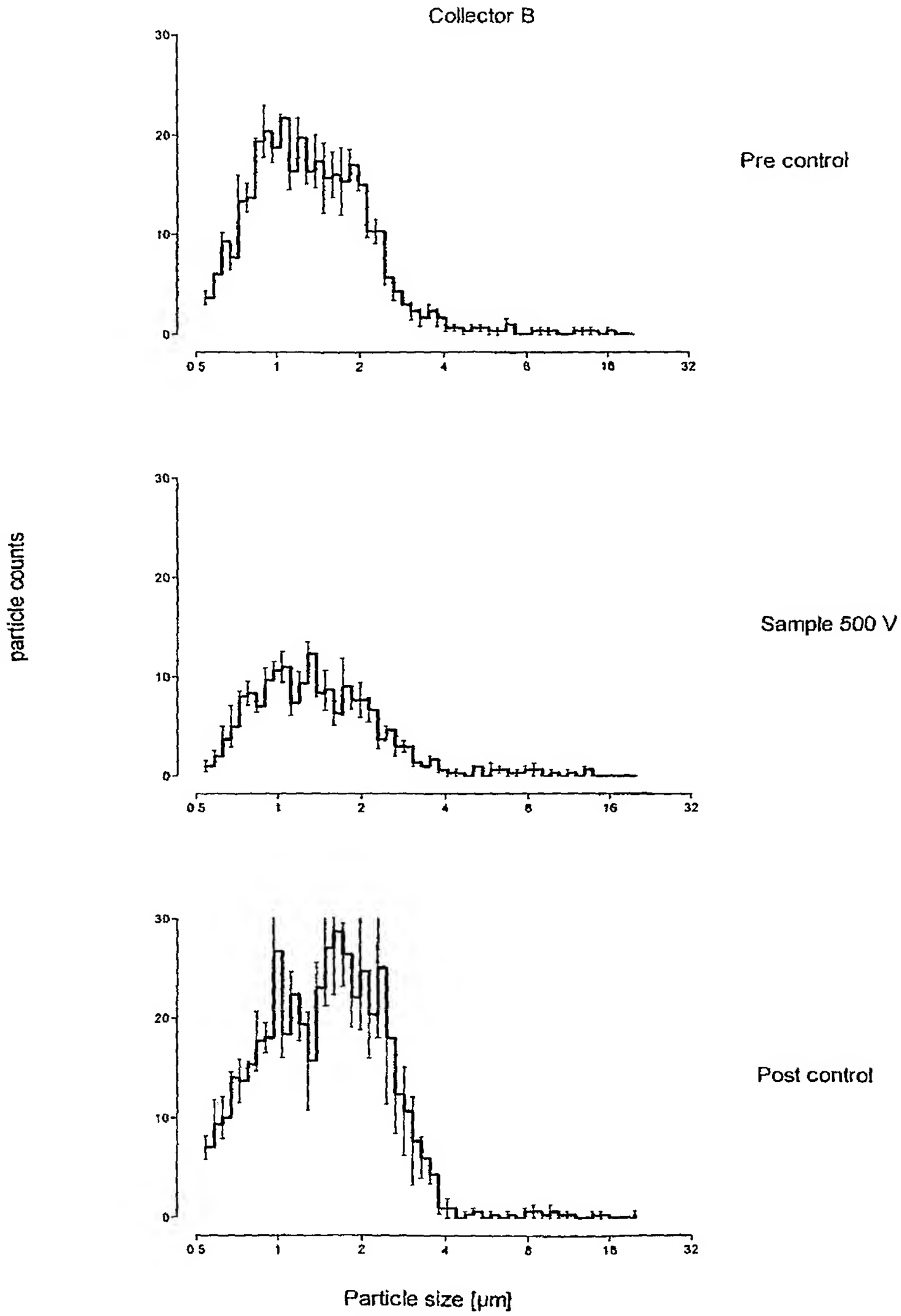

Figure 33. Chip collector B. Samples taken with 600 V difference over electrodes.

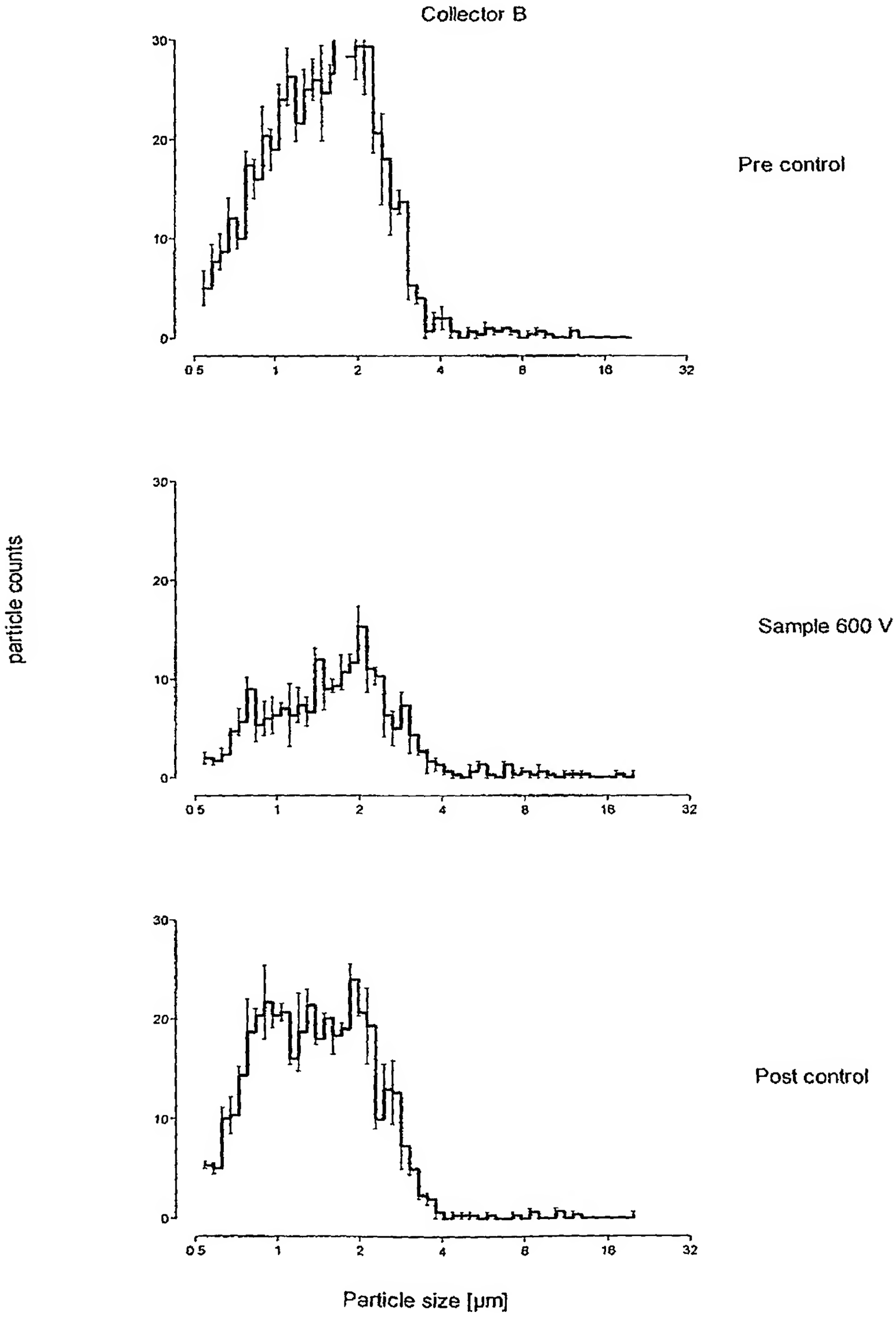

Figure 34. Chip collector B. Samples taken with 700 V difference over electrodes.

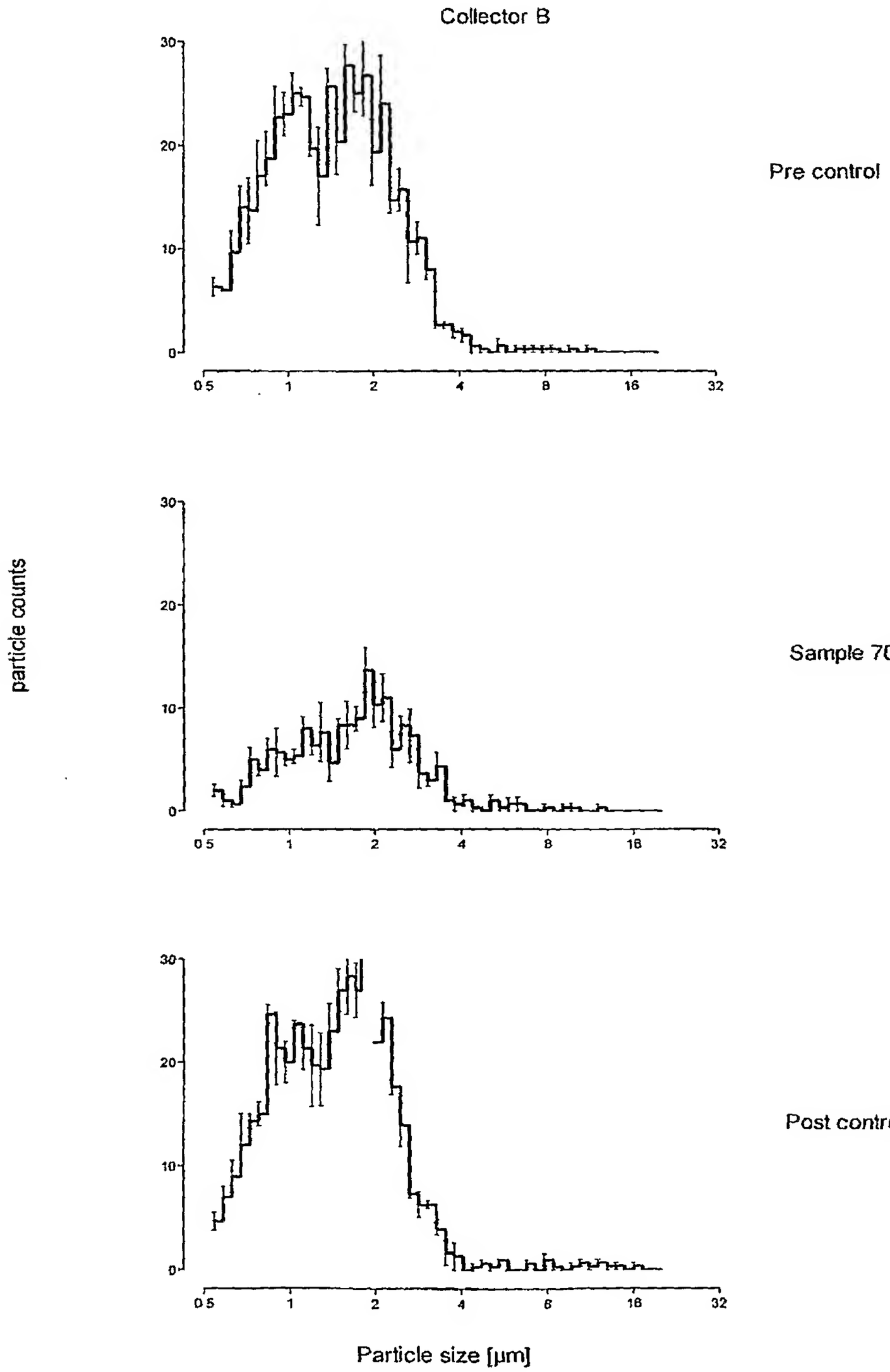

Figure 35. Chip collector B. Samples taken with 800 V difference over electrodes.

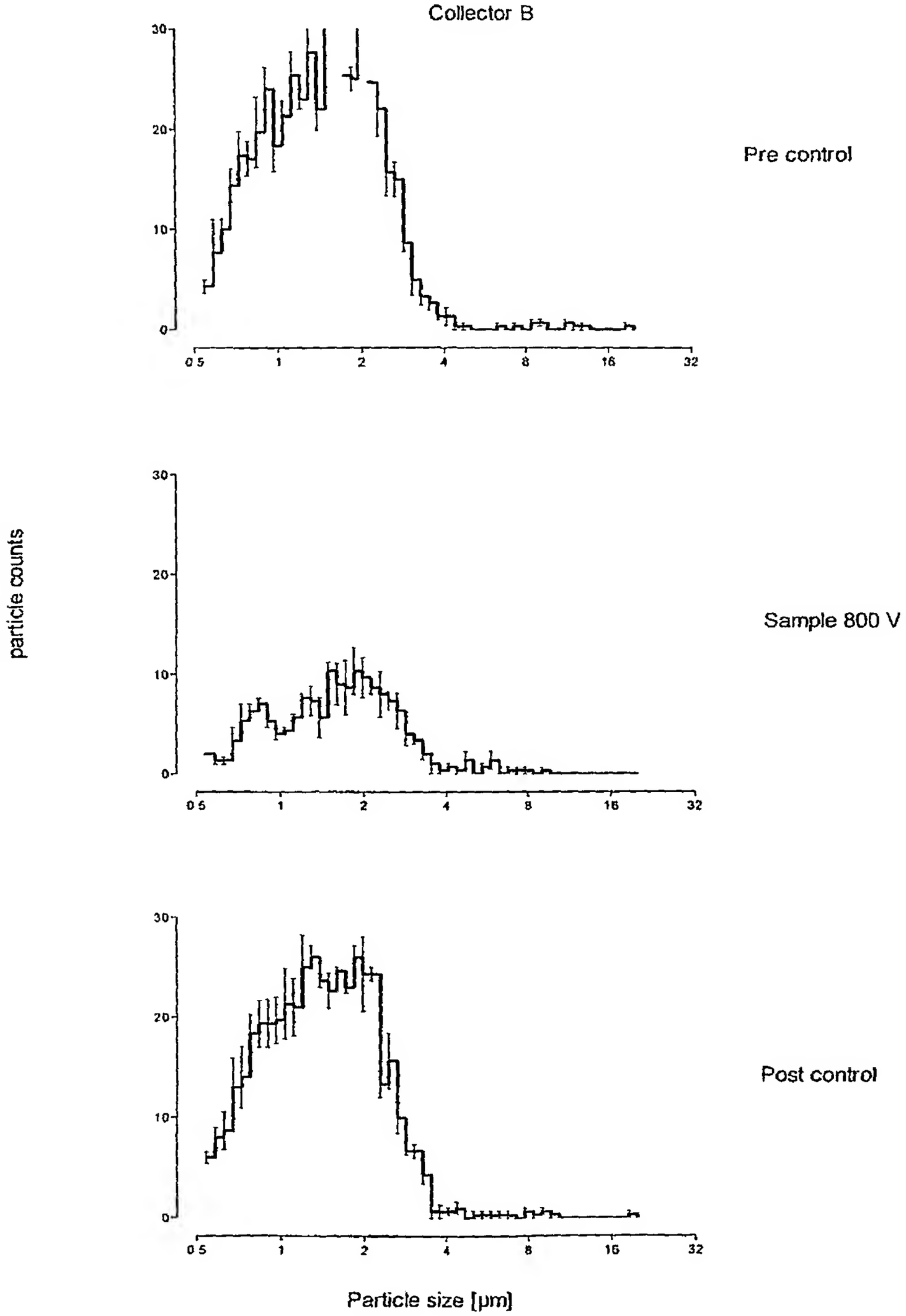

Figure 36. Chip collector B. Samples taken with 900 V difference over electrodes.

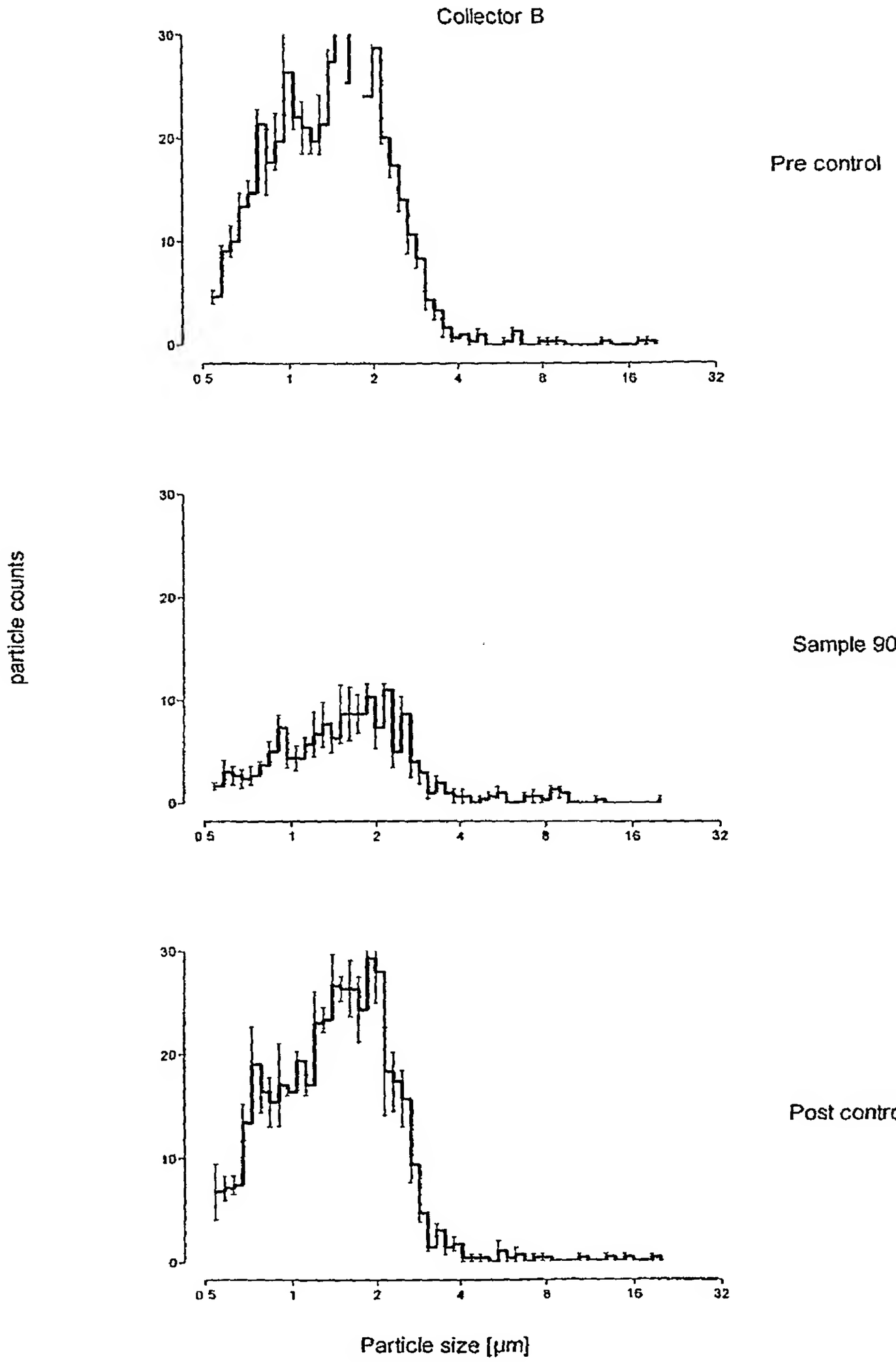

Figure 37. Chip collector B. Samples taken with 1000 V difference over electrodes.

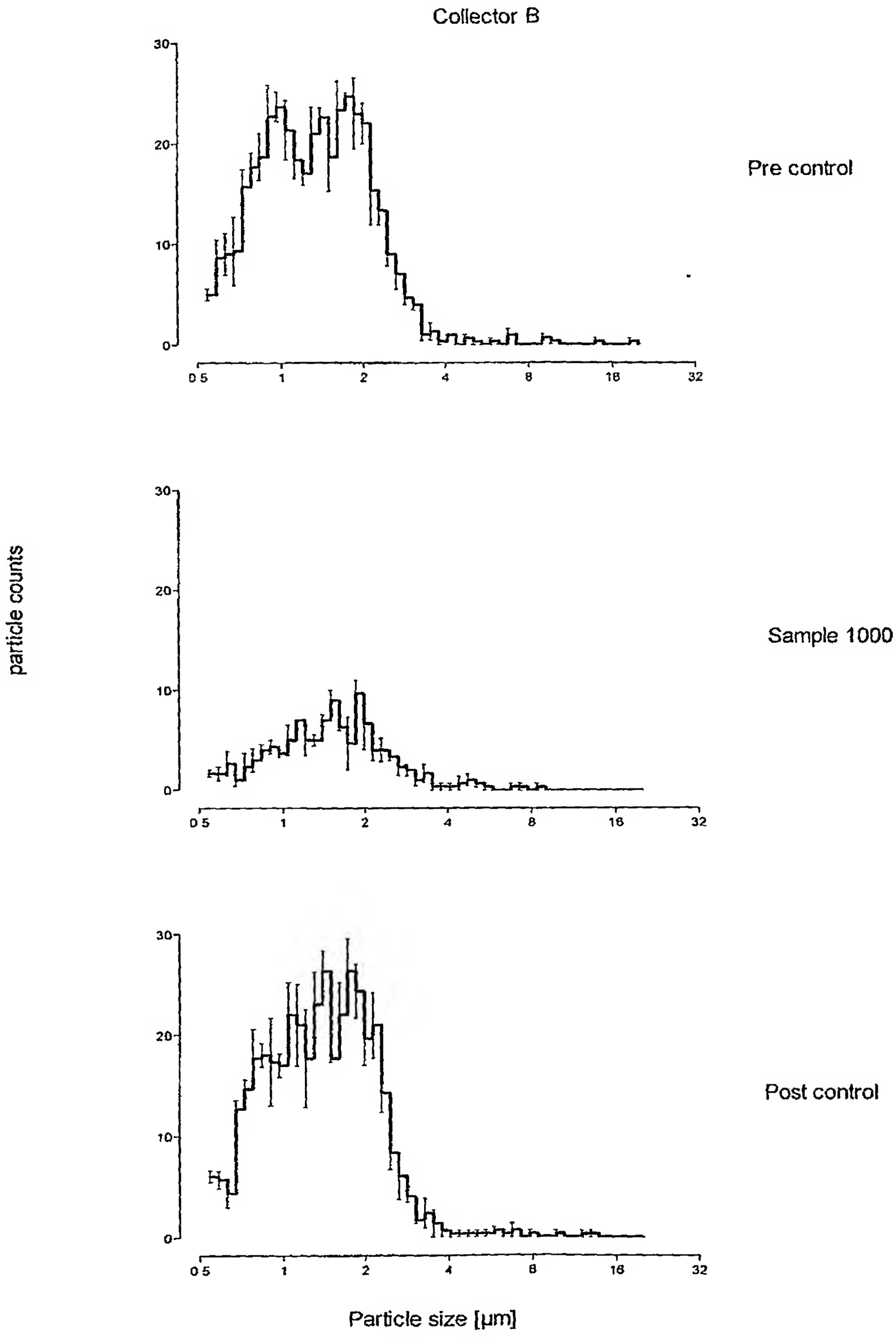

Figure 38. Chip collector B. Samples taken with 1100 V difference over electrodes.

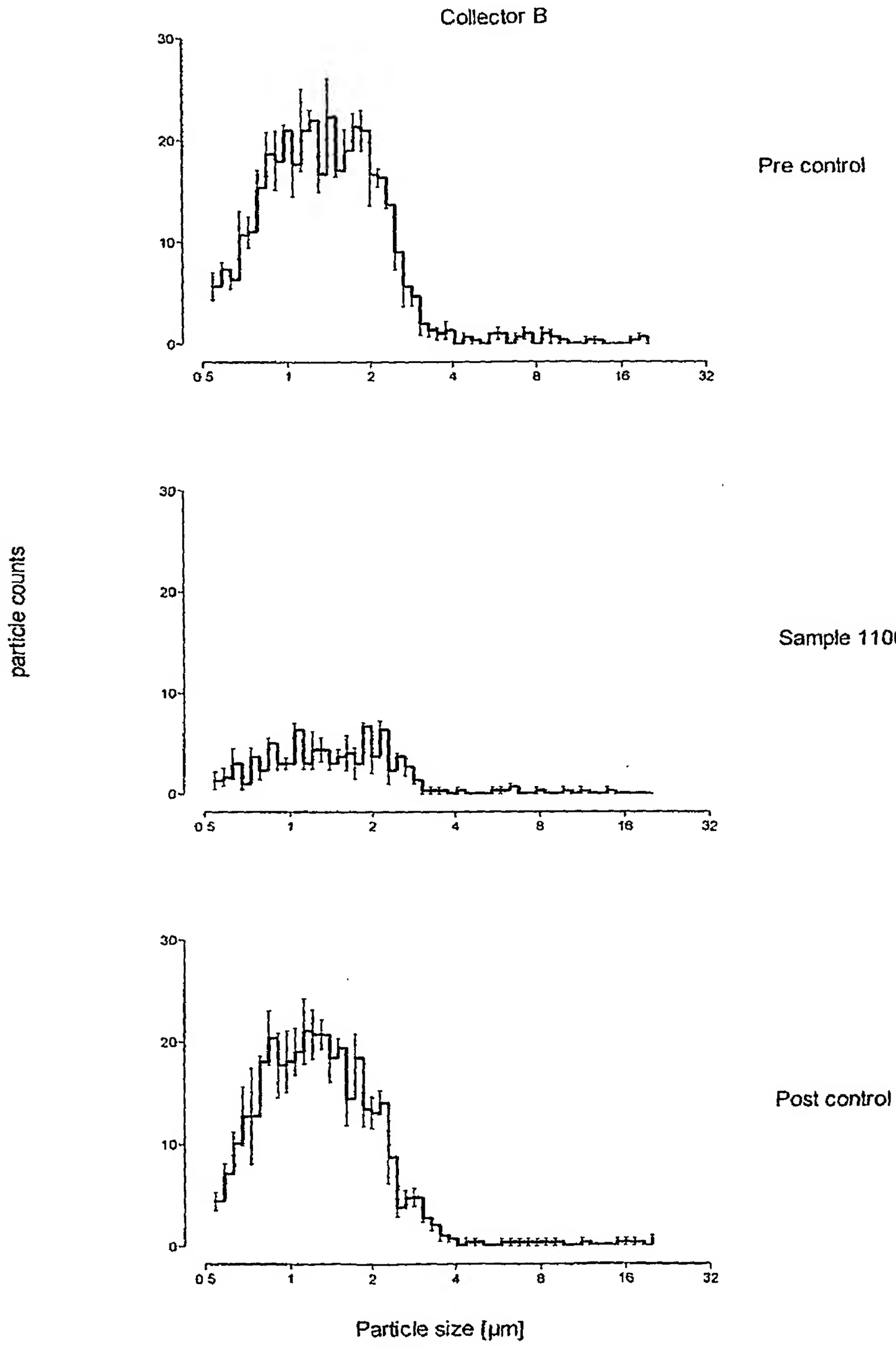

Figure 39. Chip collector B. Samples taken with 1200 V difference over electrodes.

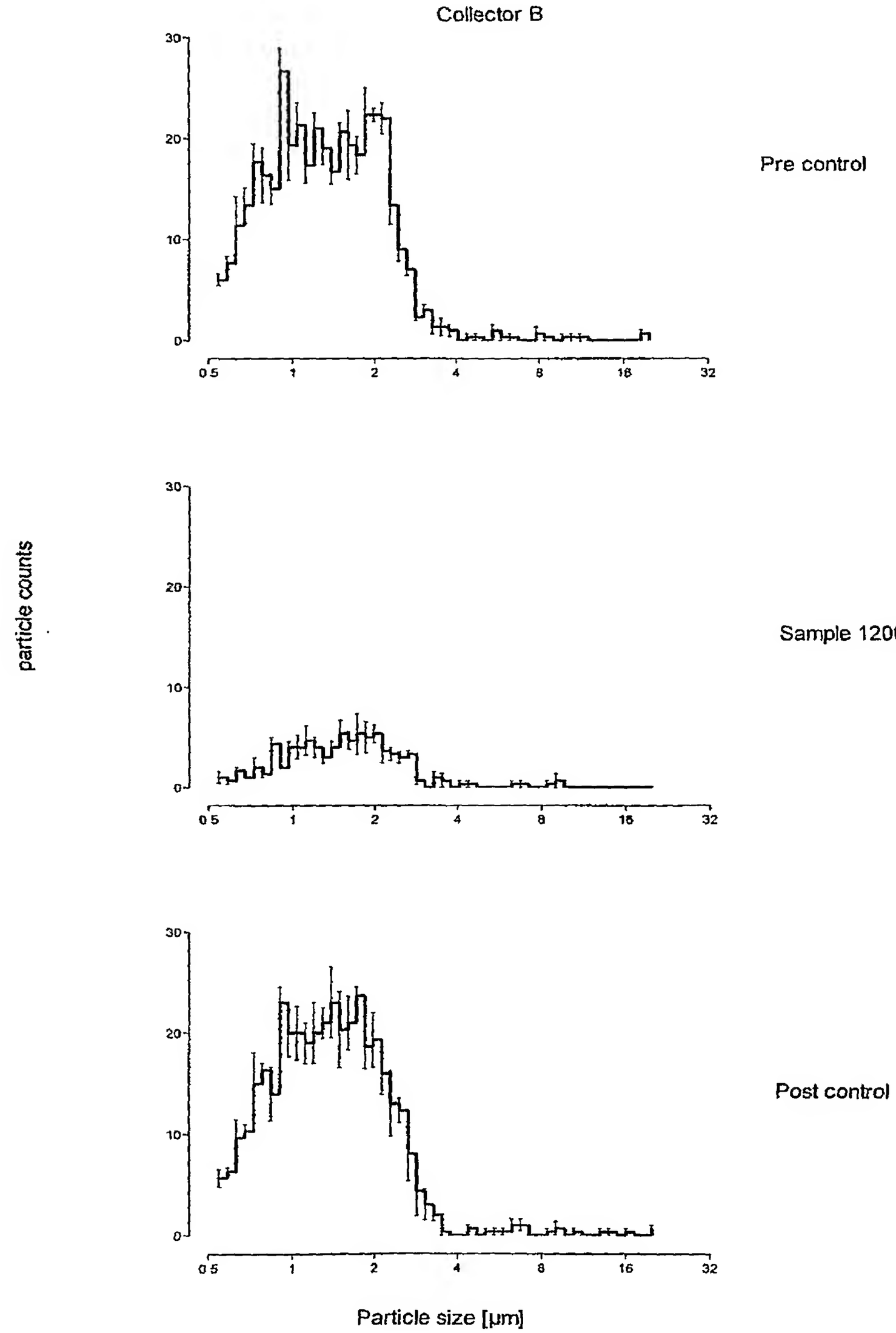

Figure 40. capture efficiency plotted versus Voltage.